INVESTIGATION OF VARIOUS BACTERIAL ISOLATES IN DEGRADATION OF SPECIFIC HYDROCARBONS

BY

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THIS WORK IS DEDICATED

TO

MY FATHER (AMIDU MALIK)

MY LATE MOTHER (NATOGMAH ALIMA)

MY WIFE (MOHAMMED BEILAWU) AND SON (MALIK KARIM KATARI)

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I am highly grateful to Allah for making my dreams come to fruition.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

LMW	:	Low Molecular Weight
HMW	:	High Molecular Weight
SPME	:	Solid Phase Micro Extraction
PDMS	:	Polydimethylsiloxane
PAH	:	Polycyclic Aromatic Hydrocarbon
USEPA	:	United States Environmental Protection Agency
SEM	:	Scanning Electron Microscopy

ABSTRACT

Full Name : Malik Karim

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Bioremediation represents an effective and environmentally benign technique for cleaning hydrocarbon contaminated sites. Biodegradation of naphthalene, phenanthrene and pyrene was studied via deploying 7 bacterial strains. Biodegradation was carried out at temperature range (10 ^{o}C – 37 ^{o}C) and pH (5.0 – 9.0) for 18 days period. The initial concentration of each Polycyclic Aromatic Hydrocarbon (PAH) was maintained at 100 ppm throughout biodegradation experiment. Solid Phase Micro Extraction with polydimethylsiloxane coated fiber was used for sample extraction, while Gas Chromatograph-Mass Spectrometry (GC/MS) was employed for detection and analysis residual PAHs.

Bacterial isolates as well as consortium biodegraded optimum quantities of PAHs between 25 ^{o}C – 37 ^{o}C, and pH values near 7.0. *Proteus mirabilis* appeared to have metabolized 95% phenanthrene. *Brevibacillus brevis* degraded about 96.5% phenanthrene at 37 ^{o}C. Bacterial consortium C_7 co-metabolized 78%, while C_4 and C_2 biodegraded over 50% pyrene. *Burkholderia fungorum* and *Rhodococcus qinshengi* utilized nearly 56% and 58% pyrene, respectively, at mesothermic temperature. Naphthalene was completely mineralized in consortium C_7, C_4 and bacterial isolates *Burkholderia fungorum* and *Rhodococcus qinshengi* inoculated media at 37 ^{o}C.

In neutral pH medium, *Rhodococcus qinshengi* and *Burkholderia fungorum* degraded over 90% phenanthrene. In the medium inoculated with *Proteus mirabilis*, approximately 95% biodegradation occurred after 18 days incubation. *Brevibacillus brevis* and *Paenibacillus borealis* both mineralized not less than 96% phenanthrene at pH 7.0. In acidic medium, 75% pyrene biodegradation transpired in flasks inoculated with consortium C_7. Consortium C_4 mineralized not less than 70%. *Burkholderia fungorum* and *Rhodococcus qinshengi* exhibited consistent metabolism across varying media pH, and degraded 59% – 61% pyrene at pH 5.0.

The bacterial strains; *Burkholderia fungorum*, *Rhodococcus qinshengi*, *Proteus mirabilis*, *Paenibacillus borealis* and *Brevibacillus brevis* are potential candidates for degradation of hydrocarbons and bioremediation of contaminated environments.

ملخص الرسالة

الاسم الكامل: مالك كريم

عنوان الرسالة: فحص العازلات البكتيرية المختلفة في تحليل بعض المواد الهيدروكربونية

التخصص: العلوم البيئية

تاريخ الدرجة العلمية: مايو 2015

تمثل المعالجات البيولوجية من التقنيات الفعالة والحميدة بيئيا لتنظيف المناطق الملوثة بالمواد الهيدروكربونية. لقد تمت دراسة تحلل النفثالين, الفيناثرين والبايرين بواسطة سبعة سلالات بكتيرية. وقد تم اجراء تجارب التحليل البيولوجي في درجات حرارة من 10 الى 37 درجة مئوية ودرجات حموضة من 5.0 الى 9.0 لمدة 18 يوما. وتم تثبيت التركيز الاولي الهيدروكربونات العطرية متعددة الحلقات عند 100 جزء من المليون طوال تجربة التحليل البيولوجي. ولاستخراج العينة تم استخدام الاستخراج المصغر مع أستخدام ألياف ثنائي ميثيل الاوكسان المغلفة. بينما تم استخدام طيف الغازالكتلي الكروماتوغرافي لكشف وتحليل الهيدروكربونات العطرية متعددة الحلقات المتبقية.

العزل البكتيري وكذلك اتحاداتها عملت على تحليل الكميات المثلى من الهيدروكربونات العطرية متعددة الحلقات المتحللة مابين درجتي 25 الى 37 درجة مئوية ودرجة حموضة 7.0. أظهرت بروتيوس ميرابيليس البكتيرية نسبة استقلاب 95% من الفيناثرين. كما ان بريفيباسيللس بريفيس قد حلل 96.5% من الفيناثرين في درجة حرارة 37 درجة مئوية. اتحاد سي-7 البكتيري خلص الي 78% استقطاب مشترك, بينما اتحادي سي-4 وسي-2 حلل اكثر من 50% من البايرين. البيركولديريا فنغورم والرودوكوكس قنشنقي استهلك حوالي 56% و 58% من البايرين, على التوالي في درجات الحرارة المتوسطة. أما النفثالين فقد تمعدن تماما في اتحادي سي-7, سي-4 والعزل البكتيري للبيركولديريا فنغورم والرودوكوكس قنشنقي عند درجة حرارة 37 مئوية.

عند الاوساط الحمضية المعتدلة فان البيرخولديريا فنغورم والرودوكوكس قنشنقي تمكن من تحليل 90% من الفيناثرين. أما في الاوساط الملقحة بالبروتيوس ميرابلس, قفد سجل تحلل بحوالي 95% بعد 18 يوما من التقليح. بريفيباسيللس بريفيس و بانيباسيللس استطاعا تمعدن 96% من الفيناثرين. أما في الاوساط الحمضية فان تحلل مقداره 75% من البايرين قد لوحظ في القوارير الملقحة باتحاد سي-7. أتحاد سي-4 ساعد على تمعدن نسبة لا تقل عن 70%. البيركولديريا فنغورم والرودوكوكس قنشنقي أظهرا معدل أيض متشابه عند درجات حموضة متفاوتة, واستطاعا تحليل 59%-61% من البايرين عند درجة حموضة 5.0.

السلالات البكتيرية؛ البيرخولديريا فنغورم, الرودوكوكس قنشنقي, البروتيوس ميرابلس, البانيباسيللس بوريالس والبريفيباسيللس بريفيس يمكن أن تكون من المواد المرشحة للاستعمال لتحليل المواد الهيدروكربونية والمعالجات البيولوجية لملوثات البيئة بصورة عامة.

CHAPTER 1

INTRODUCTION

1.1. Bioremediation overview

Biodegradation of hydrocarbons occurs spontaneously in the presence of microbes as well as favorable environmental parameters [1]. The alteration in the composition of hydrocarbon pollutants, under the influence of physical, chemical and biological factors, is invariably referred to as weathering. Bioremediation represents a viable approach to catalyze the removal of hydrocarbons from polluted environments [2][3]. Diverse microbes belonging to a variety of genera utilize hydrocarbons as primary carbon and energy source. These microorganisms are not uncommon in the environment [4]. Bacteria are considered the predominant agents of biodegradation [5]. Nonetheless, Hydrocarbons could be degraded by wide range of yeast and filamentous fungi, algae, cyanobacteria and some protozoan organisms [6][7][8].

1.2. Environmental entry of PAHs

PAHs enter into the environment essentially through petrogenic, pyrogenic and biogenic sources [9]. Petrogenic PAHs originate from petroleum and its related products,

characterized by abundance of alkyl-substituted PAHs such as alkyl naphthalenes, alkyl phenanthrenes and alkyl dibenzothiophenes. Pyrogenic PAHs arise from combustion processes and consist of preponderance of unsubstituted PAHs. Biotransformation of precursors of biogenic origin culminates in biogenic aromatic compounds such as aromatic amino acids, lignin compounds and their related derivatives. Anthropogenic (petrogenic and pyrogenic) input including oil spills, fossil fuel combustion, waste incineration, coal gasification and liquefaction processes are among the primary sources for elevated concentration of PAHs in the natural environment [9]. Primary emission sources include wood and coal burning, automobiles, heat and power plants, and refuse burning [10].

1.3. Toxicological Significance of PAHs

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic compounds that have received tremendous environmental concern as consequence of health risk associated with their persistence, potential carcinogenic, teratogenic and mutagenic properties [11][12]. Endocrine disrupting activities of PAHs is well documented. Out of over 200 PAH congeners discovered globally, 16 are identified as priority environment pollutants by the United States Environmental Protection Agency (USEPA) [13]. Given their lipophilic nature, PAHs tend to bioaccumulate in the fatty tissues of animals, and may threaten human and ecosystem health through possible bioconcentration and biomagnification in food chain [14][15]. Benzo*(a)*pyrene is known to decrease zooplankton abundance and structure of community [16]. PAHs from petrogenic origin cause edema, spinal curvature and related abnormalities in fish embryos [17]. PAHs and their metabolites pose serious

health problems in human food [18]. The effects of PAHs on estuary algal and microbial biomass was demonstrated by Nilsen et al. [19]

1.4. Properties of PAHs

PAHs compounds tend to possess remarkably low bioavailability, and are therefore categorized as hydrophobic contaminants [20]. As a consequence of their low water solubility, these pollutants are largely persistent and resist biological, chemical and photolytic decomposition [20]. Bioavailability of PAHs is enhanced at high temperatures owing to rising solubility, diffusion, and reaction rates. It is known that PAHs with higher molecular weight (HMW) have lower solubility than the lower molecular weight (LMW) counterparts, a factor which enormously inhibits the accessibility of the former to microbial assimilation and metabolism [21]. Table 1 represents a summary of physicochemical properties of model PAHs. PAHs have the tendency to undergo rapid sorption to mineral surfaces and organic matter in soil matrix, as can be seen from their LogKow values in table 1. Sorption to mineral and organic constituents could be reversible or irreversible. Irreversible sorption severely limits chemical and biological extraction of contaminants [22]. Figure 1 depicts the structure of typical LMW and HMW PAHs.

Table 1 Physicochemical properties of specific PAHs at 25 °C, Mackay and Callcott [23]

PAHs	Sw (gm^{-3})	B.P °C	M.P °C	H ($Pa.m^3/mol$)	LogKow	Ps (pa)
Naphthalene	31	218	80.5	43.01	3.37	10.4
Anthracene	0.045	339	101	3.24	4.57	0.02
Phenanthrene	1.1	340	216.2	3.96	4.54	0.001
Pyrene	0.132	360	156	0.92	5.18	0.0006

Note: Solubility in water (Sw), Boiling point (B.P), Melting point (M.P), Henry's constant (H), Octanol-water partition coefficient (Kow), Vapour pressure (Ps)

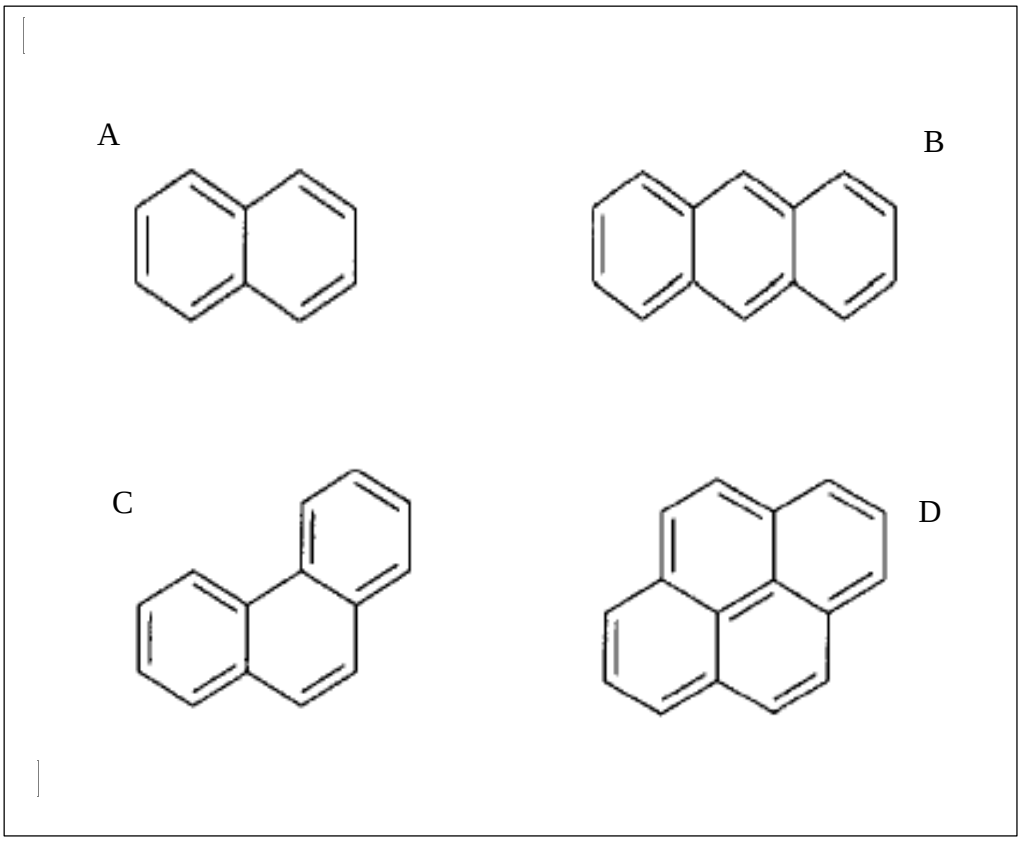

Figure.1 Structure and Molecular Weights (g/mol) of model PAHs; (A) Naphthalene, (B) Anthracene, (C) Phenanthrene and (D) Pyrene; (A) 128.2, (B) 178.2 (C) 178.2, and (D) 202.3, respectively [23].

1.5. Factors Influencing Bioremediation Kinetics

Diversity of factors tend to impact the rate of microbial pollutant uptake and concomitant degradation. Physical and environmental factors profoundly affecting the kinetics of microbial hydrocarbon degradation, include temperature, pH, salinity, oxygen, nutrients, and physicochemical composition of petroleum [24][25]. This section elaborates on the mechanics behind the impacts of the above parameters on microbial bioremediation of hydrocarbons.

1.5.1. Temperature

Temperature directly influences the rate of biodegradation and the physical and chemical characteristics of hydrocarbons, such as viscosity, solubility, diffusion rate, volatilization, mass transfer etc. Petroleum oil and its components, including their relative bioavailability change drastically as a consequence of temperature impact [26][27][28].

Investigations on the biodegradation of bunker fuel following the spillage at Chedabucto Bay, revealed that 41–85% of benzene-soluble fraction diminished within 7 days incubation at 15 ºC. Contrarily, 21–52% bio-mineralization was observed following 14 days incubation period at 5 ºC [29]. Strangely enough, ambient temperatures near 0 °C do not completely halt microbial degradation activities in seawater [30][31]. The optimum temperature for growth of hydrocarbon-degrading *Rhodococcus* strain isolated from polluted antarctic soils ranged between 25 ºC and 30 ºC [32]. Similarly, the optimum

temperature for brown dye degradation was found to be 30 °C for *Galactomyces geotrichum and Brevibacillus laterosporus* [33]. Thermophilic bacteria are adapted to mineralization at extreme temperatures. For example, between 60 – 70 °C, efficient conversion of PAHs with three to five rings by pure and mixed cultures of extreme thermophilic bacteria *Bacillus sp.* and *Thermus brockii* has been reported [28][34]. Biological degradation of PAHs and n-alkanes appeared sufficient at 0 ^{o}C and 5 ^{o}C, but declined dramatically for several components at lower temperatures [35]. At such low temperatures, psychrophilic degraders represent the probable dominant candidates in the microbial community [36].

1.5.2. Nutrients

The major limiting nutrients in bioremediation of hydrocarbon polluted environments such as water and soil is availability and distribution of nitrogen and phosphorus [37]. Biostimulation essentially entails identifying and adjusting nutrients levels in order to delimit their apparent impact on the kinetics of contaminants biodegradation by indigenous microorganism prevalent at polluted sites [38]. Nitrogen, phosphorous and potassium (N.P.K) are predominantly implicated in the cellular metabolism and successful exponential increase in microbial biomass which is vital to degradation kinetics. In hydrocarbon contaminated sites, organic carbon levels are disproportionately large leading to drastic depletion of mineral nutrients during microbial metabolism [39]. Studies has demonstrated that C:N ratio greater than 25:1 is optimal for microbial growth and ultimate

degradation of creosote [40]. Addition of N and P to increase nutrient levels and spur biodegradation has been studied by several researchers. The extent of oil biodegradation can be hindered by imbalanced C:N:P ratio resulting from elevated carbon and poor nitrogen levels generated by oil hydrocarbons due to reduced propensity of microbes to form viable biomass [41].

Supply of fertilizer heightened the rate of oil biodegradation in antarctica waters by several folds than elevated seawater temperature [41]. Sarkar et al. [42] improved the rate of hydrocarbons biodegradation up to 96% in diesel polluted soils through supplementation with bio-solids and inorganic fertilizers containing substantial amounts of N and P. Similarly, Delille et al. [31] recorded enhanced diesel oil biodegradation in antarctic coastal sea via commercial fertilizer-aided biostimulation. However, Nikolopoulou and Kalogerakis [43] warned that higher concentrations of N and P could culminate in eutrophication, encouraging algal growth and ultimately reducing dissolved oxygen concentrations in water with concomitant reduction in rates of aerobic biodegradation.

1.5.3. Salinity and pH

Salinity of the natural environment exert significant impact on the rate of microbial degradation of hydrocarbons. Olajide et al. [44] observed that rising levels of NaCl concentration in water decreases the overall effect of hydrocarbon biodegradation. The effects of pH on biodeterioration has been vividly elucidated by Wong et al. [45] who inferred that pH range of 5.5–7.5 do not significantly affect bacterial growth. However,

phenanthrene removal was 40% at pH 5.5 but exceeded 80% at pH 7.5 in 16 days incubation period. Similarly, a strain of *Sphingomonas paucimobilis* elicited high sensitivity to pH of culture media, resulting in significantly reduced degradation of phenanthrene and anthracene at pH 5.2 compared with pH 7 [46]. Maximum bacterial degradation of most PAHs tend to occur at pH 7.5 as bacterial population is evidently abundant under such pH conditions [47]. However, PAHs degradation has been reported in extremely acidic soils of pH 2 [48]. The researchers found that indigenous microorganisms in the contaminated site were capable of degrading naphthalene (50%), phenanthrene and anthracene (between 10-20%) over 28 days [48]. The pH values of given medium exert enormous influence on rhamnolipids emulsifying propensity of bacteria on hydrocarbons [49]. This finding concurs with Bai et al. [50] observation that decreasing pH caused a reduction in interfacial tension in hydrocarbons and hence enhanced the relativity solubility of hexadecane in biosurfactants with resultant rise in biodegradation rate. In related study, Lovaglio et al. [49] observed maximum rhamnolipids emulsifying ability on kerosene occurring at pH 8.

1.6. Objectives

The broader goal of this research is to examine hydrocarbon degradation efficiency of the isolated bacterial strains; *Proteus mirabilis* (T2A12001), *Burkholderia fungorum* (T3A13001), *Caulobacter sp* (T2A12002), *Ralstonia sp* (T3A13004), *Paenibacillus borealis* (T2A12003), *Brevibacillus brevis* (T2C2008) *and Rhodococcus qingshengii* (TA13008). However, the focus is intended to narrow down to the following specific objectives:

1. Evaluate bacterial isolates potential to mineralize specific PAHs
2. Assess bacterial consortium PAHs degrading efficiency
3. Identify optimum temperature and pH necessary to degrade selected PAHs

1.7. Problem statement

The increase in environmental pollution via the penetration of petroleum products into aquatic and terrestrial ecosystems has culminated in progressive degradation of environmental quality. From 1985 to 1994 about 1.4 million tons of oil spill reportedly occurred, also between 1995 and 2004 approximately 0.3 million tons of oil spill was reported [51]. The remediation of such spills requires techniques that accelerate biodegradation and /or coupling bioremediation with other available technologies [52][53].

Diverse available alternatives could be deployed for treatment of hydrocarbons from accidental oil spills. Traditionally, physical, chemical and biological methods are employed to achieve remediation of contaminated sites [53]. Amongst the various technologies identified for remedying hydrocarbon contaminated environments, biological remediation techniques proved least expensive [54]. A multitude of microorganisms possess metabolic potentials to utilize toxic organic pollutants as their source of carbon, culminating in mineralization of complex organic molecules to simpler forms such as carbon dioxide and water. Effective biodegradation of certain recalcitrant hydrocarbons have been realized through coupling of biological and non-biological techniques [52][55]. Microbial decontamination of crude oil polluted sites is cost-effective and beneficial in an environmental perspective, and is recommended as best approach towards remedying oil spill environments [54].

Indigenous microorganisms prevalent at oil polluted sites are either inhibited by contaminants toxicity or lack the required catabolic enzymes to effectively biodegrade petroleum hydrocarbons [56]. In either case, introduction of microorganisms (bioaugmentation) following nutrient enrichment escalates the efficiency and rate of degradation of petroleum hydrocarbons.

The most recalcitrant components of petroleum oil represent PAHs, asphaltenes and resins. Microbial potentials to degrade oil components appear to decline from; n-alkanes, branched-chain alkanes, branched-alkenes, monoaromatics, cyclic alkanes, polycyclic aromatic hydrocarbons and ultimately, to the asphaltenes and resins [4].

Variety of bacterial strains possess metabolic routes required for biodegradation of persistent Hydrocarbons. Some of these species include *Pseudomonas, Rhodococcus, Paenibacillus and Ralstonia,* [52][57][21]. Several different strains of microorganisms have been successfully isolated from hydrocarbon polluted sites and, are thus implicated in biodegradation and decontamination of associated polluted environments. The potential to isolate significant proportions of oil-degrading microbes from oil-contaminated environment is empirical proof that such microbes represent the most functional decontaminators in the polluted environment [44].

In this research, the metabolic activity of selected bacterial strains; *Proteus mirabilis, Caulobacter sp, Ralstonia sp, Paenibacillus borealis, Rhodoccocus qingshengii, Burkholderia fungorum* and *Brevibacillus brevis* in degradation of specific hydrocarbons and possible remediation of oil polluted sites under varying temperatures and pH is investigated. The bacterial strains were previously isolated from petroleum polluted waters in the Arabian Gulf and identified via 16S RNA. Typical hydrocarbons, including, naphthalene, phenanthrene, and anthracene and pyrene are classed as highly persistent, mutagenic and carcinogenic organic pollutants by the USEPA, hence their removal from the environment is deemed imperative [13]. It is fundamentally important to ascertain the optimum conditions and parameters required for successful bioremediation of hydrocarbon contaminated environments. The role of bacteria in the transforming these contaminants of concern into relatively simpler compounds under varying environmental parameters has been elaborated in chapter two.

CHAPTER 2

LITERATURE REVIEW

2.1. Bacteria as Degraders

The success of biological remediation efforts is hinged on the availability of active microbial community of PAHs-degraders [58]. Bioaugmentation utility is realized when potential degraders are not prevalent in the right proportions at the contaminated environment. Bioaugmentation refers to supplementing microbial degradation in environments polluted with recalcitrant toxic hydrocarbons by employing specialized microbial cells capable of biologically degrading contaminants of concern [58]. The technique is suitable for two distinct sites; (i) sites with insufficient microbial cells or (ii) where indigenous microbes lack metabolic pathways necessary to mineralize the target contaminants. Bacteria are regarded as the reliable candidates of biodegradation [5]. Most petroleum hydrocarbons are susceptible to bacterial attack and biodegradation. Nevertheless, microbial oil biodegradation pattern is widely known to decline beginning from LMW to HMW PAHs [4].

Diversity of bacterial strains possess the metabolic pathways essential for biodegradation of recalcitrant hydrocarbons. Species of *Pseudomonas, Rhodococcus, Paenibacillus and Ralstonia*, are few of the rigorously investigated bacteria for PAHs biodegradation. These strains have been identified as degraders of benzene, toluene, ethylbenzene and xylene (*Pseudomonas, Rhodococcus and Ralstonia*), and polyaromatic hydrocarbons including

anthracene (*Rhodococcus*), and benzo(a)pyrene (*Rhodococcus and Mycobacterium*) [52][57].

2.2. Degradation activity of Isolated Strains and Consortium

In practical perspective, microbial consortium is beneficial for bioremediation compared with pure culture as the former provides metabolic diversity and vitality required for real practical applications than the latter [59]. This finding concurs with Alisi et al. [60] and Li et al. [61] who confirmed significant degradation rate via the deployment of microbial consortia consisting of several different strains of diverse metabolic routes. Complex community of marine microbes comprising *Pseudomonas, Brevibacterium, Alcaligenes, Arthrobacter and Bacillus* were observed to co-metabolize persistent components of oil dominated by asphaltenes and resins. The results accentuate synergistic interactions among individual strains in the microbial community during various stages in biodegradation pathway [25]. Bacterial communities are recognized for their active cometabolism of hydrocarbons – synergistic activity. It has been recorded that phenanthrene-degrading consortium have the potential to decontaminate about 58% of phenanthrene in a week [62].

2.3. Hydrocarbon degradation activities of isolated strains

Mohite et al. [63] found that *Citrobacter freundi and Proteus mirabilis* isolates are effective for bioremediation of phenol polluted sites. These bacterial strains biodegraded approximately 90% of 100 mg/L phenol within 80 hour as major source of carbon and energy with the lag phase increasing proportionately with phenol concentration. In a related study, Mohana et al. [64] realized that a consortium of *Pseudomonas aeruginosa, Stenotrophomonas maltophila and Proteus mirabilis* possess the capability of decolorizing and degrading anaerobically treated distillery spent wash. *Proteus mirabilis* strain, was identified as the strain catalyzing decolorization and biodegradation of a sulphonated reactive azo dye from a municipal dump soil close to Lagos, Nigeria. The strain effectively decolorized 100 mg/l of the dye in time span of 5 hours [65]. Olajide et al. [44] investigated the degradation ability of *Proteus vulgaris* bacterium strain and concluded that the bacterium utilizes hydrocarbons as its prime carbon source and degraded kerosene, diesel and Bonny light crude oil and media at about 79, 73.8 and 78% respectively after 96 hours, with 1.0% NaCl (w/v). Investigations carried out in two petroleum polluted *Spartina* salt marshes in the New York/New Jersey Harbor confirmed the dominance of aerobic PAHs mineralizing microbial communities comprising *Pseudomonas and Paenibacillus* genera [66]. Metabolism of xenobiotics by *Paenibacillus sp* ISTP10 was reported [67].

Cultures of *Exiguobacterium sp and Burkholderia cepacia* were found to utilize diesel oil as main substrate and exhibited uniform degradation rates for *n*-alkanes from C12 to C26 in including pristane [68]. In the *rhizosphere*, association of *Burkholderia xenovorans* with plants enhanced degradation of polychlorinated biphenyls [69][70]. The activity of

Burkholderia sp in transformation of dibenzothiophene via the oxidative route termed as the kodama pathway and the utilization of fluorene, naphthalene and phenanthrene as carbon and energy sources has been reported [71]. Microbial degradation of seven commercial textile dyes made of variable structures and color characteristics by *Brevibacillus laterosporus* had been testified [72]. Total degradation of methyl orange was realized within 3hours and 9 cycles through the deployment of *Brevibacillus laterosporus* [73][33]. Ye et al. [74] demonstrated biosorption and degradation of tryphenyltin by *Brevibacillus brevis.*

In soils contaminated with crude oil *Caulobacter*, *Ralstonia, and Burkholderia sp* were among bacterial strains producing and accumulating polyhydroxyalkanoic acid whilst utilizing sodium gluconate or sodium octanoate as their principal carbon source [75]. The genera of *Ralstonia* is reported to degrade several different xenobiotics containing hydrocarbons [76][77]. For example, *Ralstonia pickettii* is known for degrading toluene, benzene, and related alkylaromatic hydrocarbons [78][79][80]. Similarly, *Ralstonia eutropha* is capable of metabolizing benzene, toluene, ethylbenzene, and xylenes as primary source of carbon [81].

The genus *Rhodococcus* possess metabolic potential to degrade broad spectrum of organic compounds, not excluding recalcitrant and toxic hydrocarbons owing to a range of diverse catabolic related genes and their robust cellular physiology [82]. *Rhodococcus erythropolis* cells contain diversity of enzymes hence, are capable of carrying out tremendous bioconversions and degradation activities [52]. De Carvalho [83] confirmed the adaptation and degradation of C6 – C16 n-alkanes and alcohols by *R. erythropolis cells* at 4 – 37 ^{o}C, pH 3 – 11 and, salts of sodium chloride and copper sulfate. A similar study executed by de

Carvalho and da Fonseca [52] on *Rhodococcus erythropolis* cells demonstrated the strain's propensity to metabolize C5 – C16 hydrocarbons as principal energy and carbon sources at 15 and 28 ^{o}C. Nearly complete degradation of cyclo-alkanes and aromatic components of fuel oil was observed after 9 months incubation. However, 60% was mineralized within the first three months of biodegradation. These observations has been accentuated by Dean-Ross et al. [85] whose findings revealed to a large degree, the utilization of anthracene, phenanthrene, pyrene ,and fluoranthene as primary carbon and energy source by *Rhodococcus sp*. The pathway for degradation of anthracene was noted as ring-fission involving double ring-cleavage pathways.

Huang et al. [86] demonstrated that augmenting Seawater with $(NH4)_2SO4$, Na_2HPO_4 and yeast extract escalates rate of biodegradation from 12.61% to 75% in 7 days at 15 ^{o}C mediated by *Rhodoccocus erythropolis*. Quek et al. [87] reported that free and immobilized *Rhodococcus* cells degraded approximately 90% of the total n-alkanes in Arabian light crude and Al-Shaheen crude tested within 1 week at 30 ^{o}C. In a similar observation, *Rhodococcus sp* isolated from crude oil-contaminated sediments degraded PAHs such as phenanthrene, pyrene , and benzo(a)pyrene as principal carbon and energy sources [88].

2.4. Degradation of specific PAHs

Several different bacterial strains possess specific PAH degrading enzymes that are discriminative, and hence degrade certain hydrocarbons efficiently at the expense of others, either in a mixture or pure form [89]. Researchers such as Biosci et al. [90] discovered microbial degradation of fluoranthene within sediments of the Arabian Gulf. *Bacillus circulans* and *Alcaligenes faecalis* were identified as the predominant degraders in the environment, removing as high as 73.4 and 71% fluoranthene, respectively. The preponderance of microbes in mangrove sediments and consequent degradation of PAHs has been reiterated [91]. Research has revealed that certain strains of bacteria are highly capable of mineralizing between 80 - 96% of pyrene [92]. The utilization of n-alkanes, phenol, anthracene and pyrene at 15 ^{o}C in the presence of *R. erythropolis* strain has been reported [93].

Dhote et al. [94] detected exponential increase in viable cell count and dry biomass of a mixed community comprising two strains and, chrysene as primary source of carbon. Additionally, biosurfactant production was shown as surface tension of the growth medium dramatically declined. Biomineralization of chrysene in the presence of non-ionic surfactants and PAHs solubilizers has been reiterated through the findings of Dave et al. [64]. In a related investigation, Kafilzadeh et al. [96] reported degradation of chrysene in petroleum polluted site in Iran. The most successful degrader in the microbiota community was noticed to be *pseudomonas putida,* as it mineralized substantial quantum of chrysene. *Pseudomonas sp* has also been implicated in the degradation of phenanthrene [97][98]. Degradation of PAHs, an assemblage of highly persistent components of petroleum oil, has

been achieved in many instances through coupling bacteria with fungi [99] [100]. A fungus, *Armillaria sp*, could degrade approximately 77% of chrysene within 30 days via deploying an arsenal of ligninolytic and dioxygenase enzymes [101]. The fungus is also characterized by its inherent tendency to attack and oxidize diverse PAHs. For example, Hadibarata and Kristanti [102] emphasized the participation of *Armillaria sp* in degradation of fluoranthene. Assimilation of fluoranthene by *Rhodococcus sp* BAP-1 has been demonstrated [103].

Adsorption-biodegradation studies revealed the capacity of *Pseudomonas aeruginosa* to utilized naphthalene as its sole carbon source [104]. *Streptomyces sp* is known to grow successfully on diverse hydrocarbons including naphthalene, phenanthrene, pyridine and cyclohexanes, using them as prime carbon source [105]. At 30 ^{o}C, *Bacillus fusiformis* is capable of decontaminating about 99.1% of the initial amount of naphthalene within 96h [106]. Bioavailability of PAHs in polluted site is a critical determinant of kinetics of biodegradation. Naphthalene solubilized by micelles in liquid media enhances their bioavailability and biological attack by bacteria [107].

Investigations carried out in mangrove sediments uncovered the degradation of phenanthrene in a mixture PAHs by a complex community of indigenous bacteria. The strain, *Burkholderia sp,* was noted as one of the active participants in mineralizing and catalyzing the ultimate disappearance of phenanthrene [108]. Moscoso et al. [109] demonstrated phenanthrene degrading efficiency by *Pseudomonas stutzeri* under aerobic conditions in flask and stirred tank bioreactors. Abd-Elsalam et al. [110] inferred that *Escherichia coli*, *Alcoligenes* sp., and *Thiobacter subterraneus* are efficient isolates for successful transformation of anthracene and phenanthrene. Research has proved microbial

consortium propensity to mineralize phenanthrene in the presence of other petroleum hydrocarbons, highlighting the possibility of achieving efficient decontamination via bioaugmentation [111].

Unlike most LMW PAHs, Benzo (a) pyrene (BaP) is regarded as a recalcitrant PAH with few bacteria capable of transforming it into simpler metabolites. Biosurfactant producing *Rhodococcus* and *Pseudomonas* are however well known to degrade sufficient proportions of BaP in polluted sites via catabolic enzymes [112]. Strain, *Arthrobacter oxydans*, efficiently mineralized BaP between 20 to 37 ^{o}C following nutrient addition [113]. Interestingly, it is reported that marine denitrifying bacteria are capable of completely degrading BaP under anoxic conditions [114]. Under similar environmental conditions, efficient mineralization of phenantherene and anthracene triggered by denitrifying bacteria has been recorded [115] [116].

2.5. PAHs Metabolism Pathways and Metabolites

Metabolic pathways of PAHs degradation have been extensively studied [117][118]. Microbial metabolism of PAHs initiates with incorporation of oxygen atoms into the benzene ring by dioxygenase enzymes culminating in the formation of cis-dihydrodiol [119], which then undergoes rearomatization by the action of dehydrogenase enzymes to yield dihydroxylated intermediates metabolites. Subsequent ring fission of the dihydroxylated intermediates may ultimately form corresponding intermediate metabolites prior to complete mineralization [120]. Variety of intermediates metabolites ranging from

simple to complex analogues could be generated depending on the metabolic pathway as well as the bacterial strain employed for degradation. For instance, a certain *Stenotrophomonas maltophilia* strain utilizing phenanthrene as its prime carbon and energy source from creosote-polluted sites at Hilo, Hawaii, liberated over twenty metabolites of phenanthrene [121].

Research has shown that phenanthrene hydroxylation and subsequent mineralization is probable through the 1,2-,3,4- and 9,10-carbon positions. Nevertheless, the predominant metabolic route is 3,4-dioxygenation predominant in most bacterial strains, contrarily, certain reports point to 1,2 and 9,10-hydroxylation [122][123][124]. For example, *Burkholderia sp* previously believed to degrade phenanthrene via 3,4-dioxygenation [125][126][127], is presently known to deploy both 3,4- and 1,2-dioxygenation routes [128][129]. The accumulation of 1-hydroxy-2-naphthoic acid, a metabolite of phenanthrene biotransformation, via metabolic activity of bacterial consortium is well established [62]. Bacterial degradation of anthracene is reported to follow 3-hydroxy-2-naphthoic acid and 2, 3-dihydroxynaphthalene routes. These intermediates are further degraded via naphthalene pathway. Such a degradation route is characteristic of species from *Pseudomonas, Sphingobium, Nocardia, Rhodococcus, and Mycobacterium* genera [130][131]. In certain bacteria, naphthalene degradation occurs via 1, 2-dioxygenation [132]. Interestingly, Morasch and Annweiler [133] documented 2,3-dioxygenation of naphthalene in *Bacillus thermoleovorans*.

The HMW PAHs such as pyrene degradation initiate with dioxygenase attack on the 4-5 carbon positions to form dihydrodiols [134] [135][136]. The pathway proceeds with ortho ring cleavage eventually producing phenanthrene-4,5-dicarboxylic acid. The pathway

segregates in succeeding steps, one entering phenanthrene pathway whilst the other catabolize the intermediate to substituted biphenyls [137][138]. Phthalic acid becomes the common intermediate product of both pathways. It is worthy to emphasize that the initial angle of attack on pyrene is at the 4-5 carbon positions, however Kim et al. [137] confirmed dioxygenation at the 1-2 carbon positions yielding a dead-end metabolite, 1,2-dimethoxypyrene.

The metabolites of naphthalene, the simplest PAH, such as naphthalene dihydrodiols, are highly bioavailable and potentially toxic compared with the naphthalene precursor [139][140]. PAHs degradation and consequent accumulation of oxy-PAHs, such as PAH-ketones, quinones and coumarins has generated a great deal of concern as they are more persistent and toxic to human health than the precursor parental PAHs [141]. These compounds may also be liberated via chemical oxidation and photo-transformation of PAHs [142][143].

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials and Microorganism

The bacterial strains previously isolated from oil contaminated sites and identified via 16S RNA were trialed for their hydrocarbon degrading efficiency. These strains were isolated, characterised, identified and preserved from previous research carried out in the King Fahd University of Petroleum and Minerals Life Science department laboratory. The strains comprise, *Proteus mirabilis* (T2A12001), *Burkholderia fungorum* (T3A13001), *Caulobacter sp* (T2A12002), *Ralstonia sp* (T3A13004), *Paenibacillus borealis* (T2A12003), *Brevibacillus brevis* (T2C2008) *and Rhodococcus qingshengii* (TA13008). The strains were activated via preculture using nutrient agar (Yeast extract, 2.0g/L; Meat extract, 1.0g/L; Peptone,5g/L; NaCl, 5.0g/L and Agar,15g/L). The strains, being transfer into nutrient broth were monitored until (OD600 nm) equalled 1.0. Bacterial cells were then harvested after centrifuging at 4000 rpm for 10 minutes. Naphthalene, phenanthrene and pyrene were of analytical grades. Bushnell Haas Minerals Medium (BH) was prepared following Bushell and Haas [178] protocol. The BH composition consists of the following ingredients (g/L): $MgSO_4$, 0.2; $CaCl_2$, 0.02; KH_2PO_4, 1.0; K_2HPO_4, 1.0; NH_4NO_3, 1.0 and $FeCl_3$, 0.05. The pH was adjusted to approximately 7.0 ± 0.2 using phosphate buffer solution. The resulting media was finally sterilized through autoclaving at 121 ^oC for 15 minutes.

3.2. Biodegradation Experiment

The study focused on environmental and biological parameters - the variable temperature ranges considered and the different bacterial strains trialed, respectively. The research investigates bacterial biodegradation on laboratory scale under three (3) different temperatures ranges; 10 ^{o}C, 25 ^{o}C and 37 ^{o}C. Effects of hydrogen ions concentration (pH) on the bacterial strains hydrocarbon mineralizing potential was also examined under media pH values of 5, 7 and 9. The strains are trialed as (a) collective consortium and, (b) individually inoculated on media spiked with specific hydrocarbons. The consortia combinations compose of; C_2 (*P. mirabilis + P. borealis*), C_4 (*Caulobacter sp + B. fungorum + P. mirabilis + P. borealis*) and C_7 (all strains). The PAHs were dissolved in *n*-hexane ($10gL^{-1}$). Erlenmeyer flask (250ml) held liquid bushnel media (BH) with 97ml mineral salts for incubation at chosen temperatures and pH. Each medium being spiked with 1ml of specific hydrocarbon, received 2ml inoculum suspension. The resulting initial concentration of individual PAHs in each flask thus reached 100 ppm [144]. Flasks were incubated in Shake-Incubator; WiseCube Fuzzy System (model WIS-20) for 18 days with continuous shaking at 120 rpm. The set-up comprised 2 replicates and a control. The control samples did not contain bacterial cells so as to account for abiotic loss of spiked hydrocarbons. Figure 2 depicts biodegradation experimental design and treatment conditions.

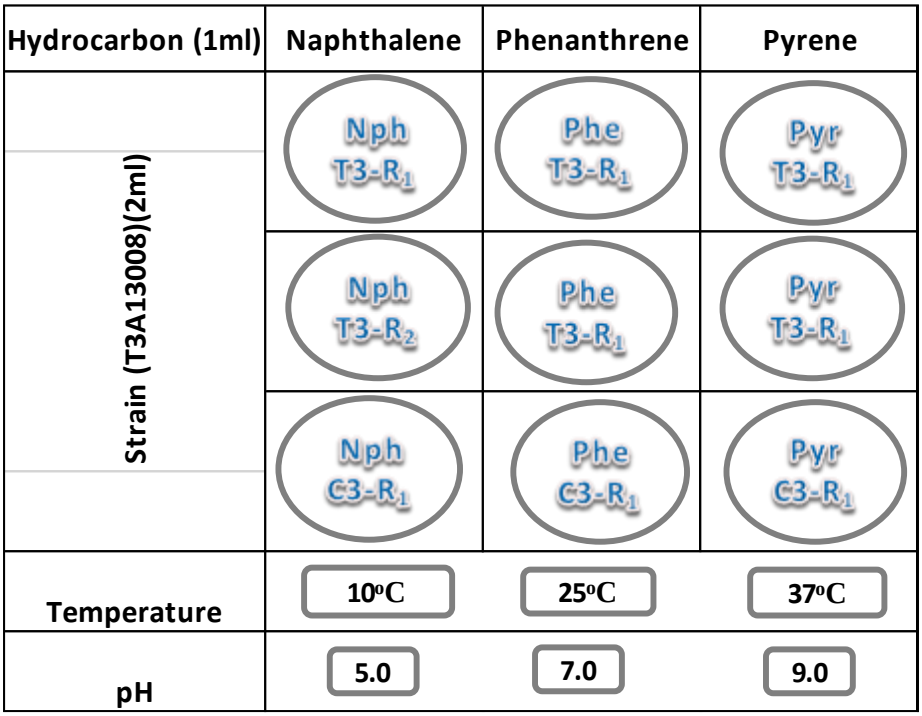

Figure 2 experimental design and treatment conditions

3.3. Analytical Techniques

3.3.1. Sample extraction and preparation

Extraction of residual PAHs and corresponding metabolites was carried out in defined intervals to measure the degradation potentials of each pure culture as well as consortium. Solid Phase Micro Extraction (SPME) was deployed for sample extraction and preparation prior to analysis for residual PAHs and related Metabolites. The SPME was coated with polydimethylsiloxane (PDMS) of 100µm thickness. SPME is a simplified and "green" technique for preparation and pre-concentration of sample without employing any solvent. SPME is an equilibrium extraction technique where fibers are immersed in the sample and target analytes in aqueous samples extracted as the analytes partition between the aqueous matrix and the fibre coating [145][146][147]. The SPME device after being immersed in samples with agitation (magnetic stirring), was allowed to equilibrate in 30 and 45 minutes for LMW and HMW PAHs, respectively [148].

3.3.2. GC-MS Analysis of Residual Hydrocarbons

Analysis and detection of unutilized PAHs was achieved using gas chromatography/mass spectrometry (GC/MS). The GC system model specifications are; Agilent Technologies (series 6890N), Injector unit (series7683B) and MS unit; inert XL EI/CI MSD (series 5975B). After the extraction attained equilibrium, the SPME fiber was inserted into the hot injection-port where the analytes desorbed into the GC column. The GC/MS method adopted is in line with King et al. [148], though marginally modified. Desorption time of

10 minutes enhanced analyte removal from the PDMS coated fiber. The fiber was conditioned (after each extraction) to limit effects of analyte carryover following successive extractions. Conditioning of the SPME device was achieved by inserting the fiber into the GC inlet port at 250 ^{o}C for 10 minutes. GC/MS temperature was programmed as follows; Inlet temperature set at 220 ^{o}C to optimize desorption. Initial oven temperature was set at 50 ^{o}C, rump to 100 ^{o}C in 15 ^{o}C/min, hold time of 2mins, rump to 200 ^{o}C at 7.5/min and ultimately increased to 300 ^{o}C.

CHAPTER 4

RESULTS

4.1. SEM analysis of bacterial strains

Scanning Electron Microscopy (SEM) was carried out to identify the various shapes of bacterial cells. The SEM enabled establishment and confirmation of the presence of different bacteria in the various media in which they were cultured. The strains are morphologically different on nutrient agar plates, however SEM images showed that all the bacteria are rod shaped, but with varying sizes. Figures 3(A-D) and 4(A-C) illustrate SEM images and photograph of bacteria on nutrient agar plates.

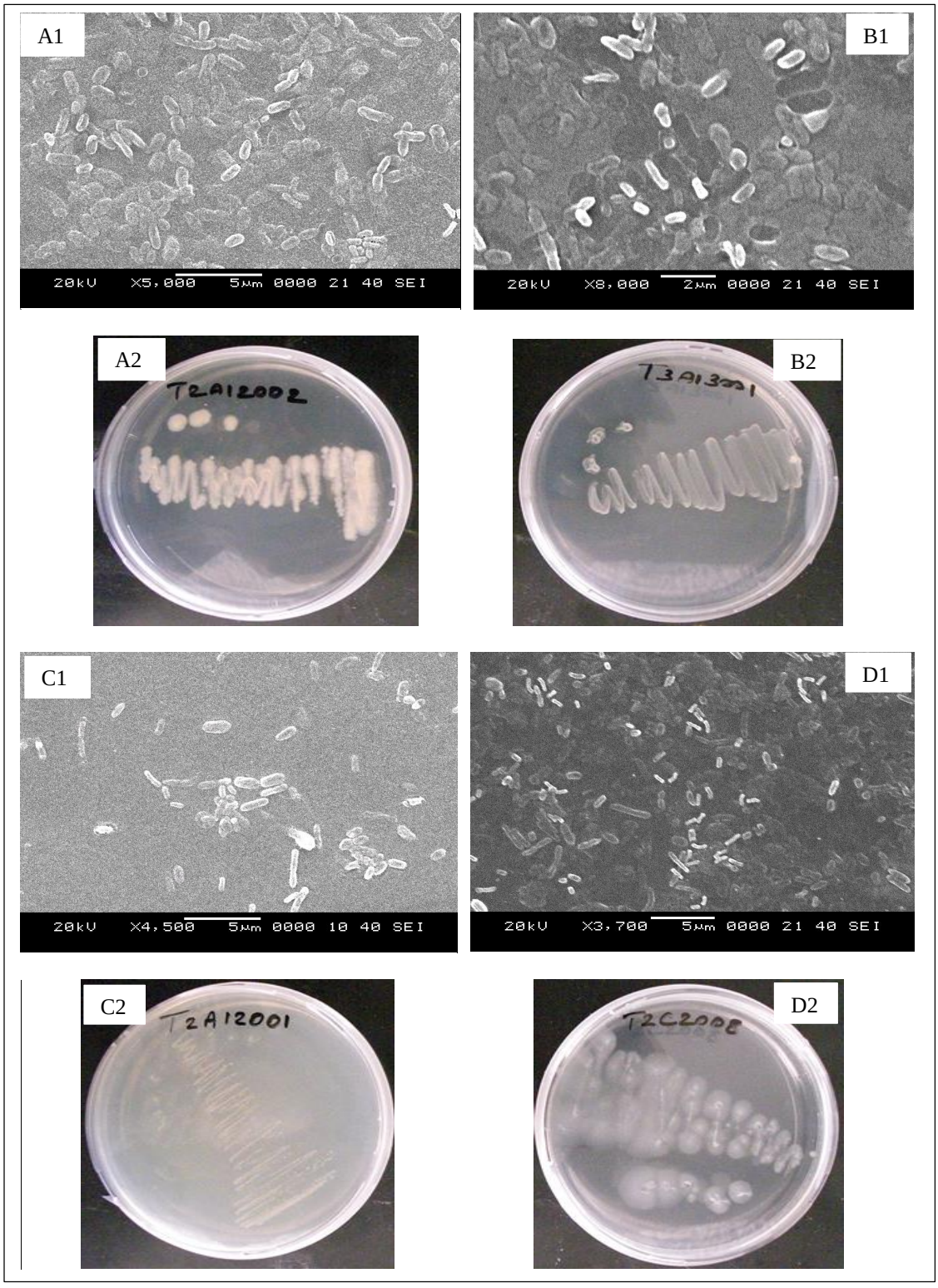

Figure 3 SEM images and photograph of bacterial colonies on agar plates*; Caulobacter sp* (A1, A2), *B. fungorum* (B1, B2), *P. mirabilis* (C1, C2) *B. brevis* (D1, D2)

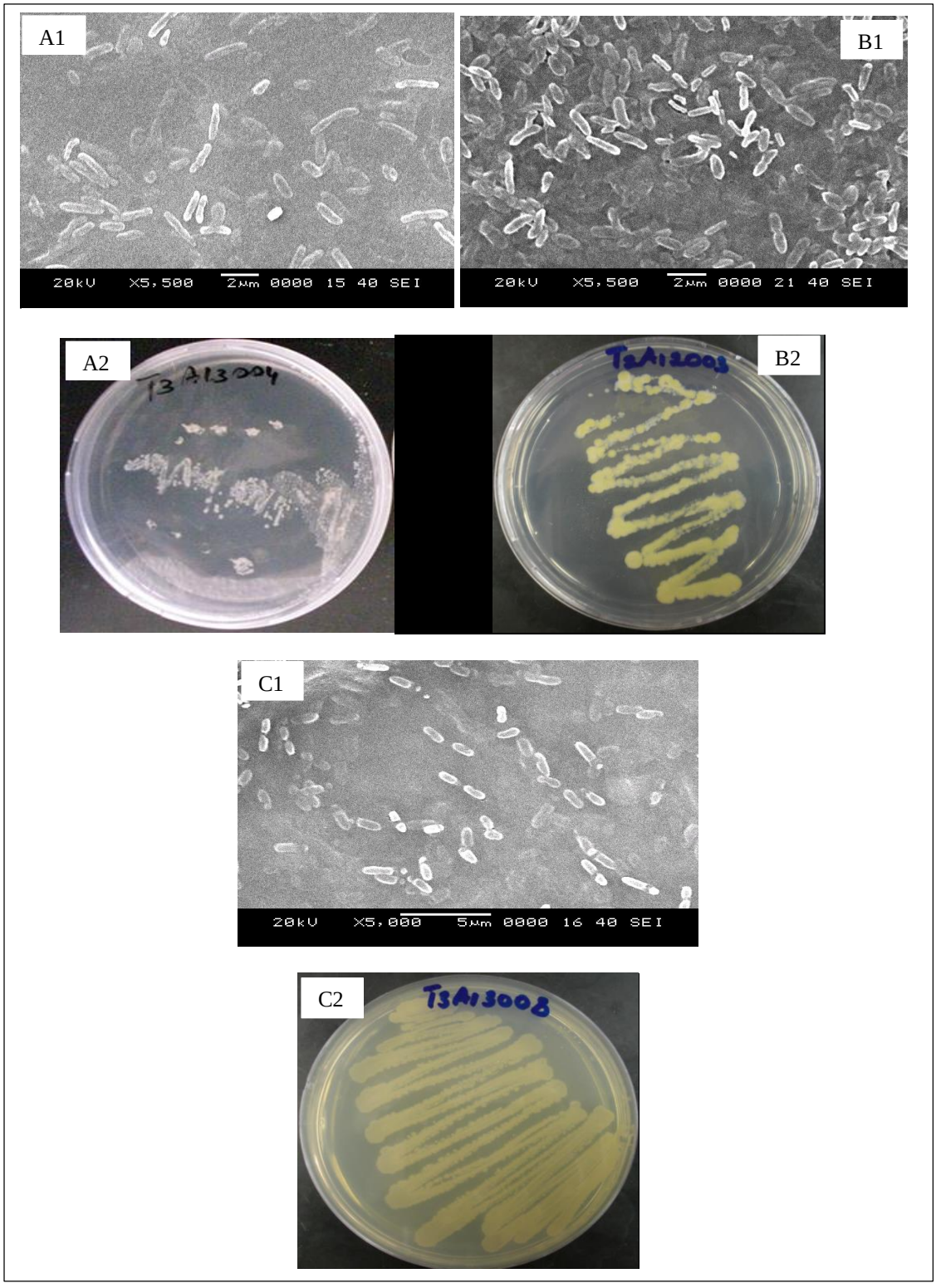

Figure 4 SEM images and photograph of bacterial colonies on agar plates*; Ralstonia sp* (A1, A2) *P. borealis* (B1, B2) *and R. quinshengi* (C1, C2)

4.2. Detection of residual PAHs

In this investigation, samples spiked with PAHs were subjected to GC/MS analysis at the end of biodegradation experiment. Figure 5 shows GC chromatogram and mass spectrum of phenanthrene. As can be seen from figure 5, GC retention time for the hydrocarbon was 13.7 mins. The molecular ion (M+) at m/z corresponded to 178.0 with major fragment ions (M+) at m/z (152 and 89). The GC elution time for pyrene was reckoned at 18.51 mins (figure 6). Molecular ion at m/z was 202.1, and fragment ions at m/z (101.0 and 174.0). Naphthalene was determined at retention time 9.53 mins (figure 7). Molecular ion was at m/z 128.0, major fragment ions at m/z being (102.0 and 64.0).

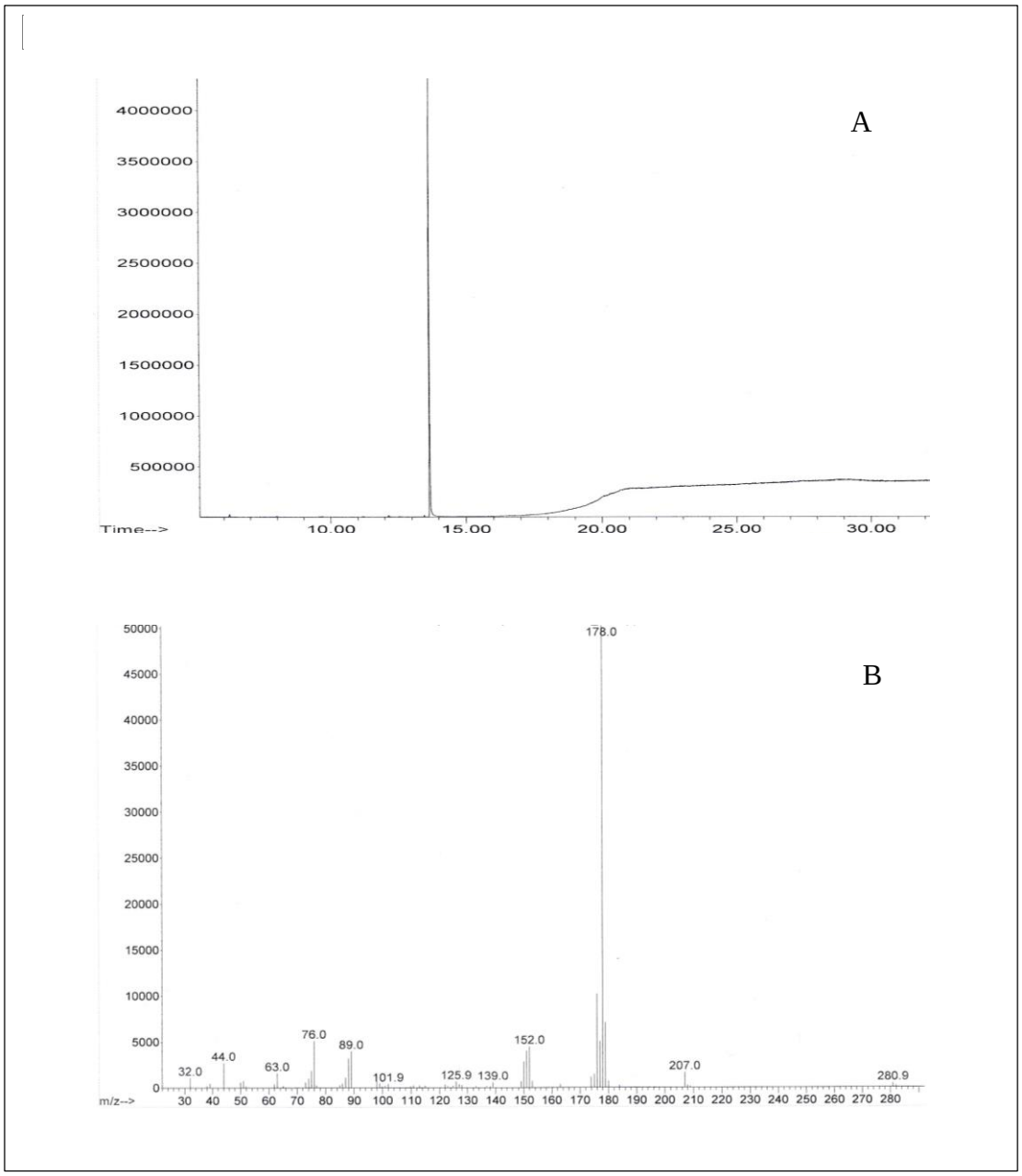

Figure 5 GC Chromatogram (A) and Mass spectrum (B) of phenanthrene

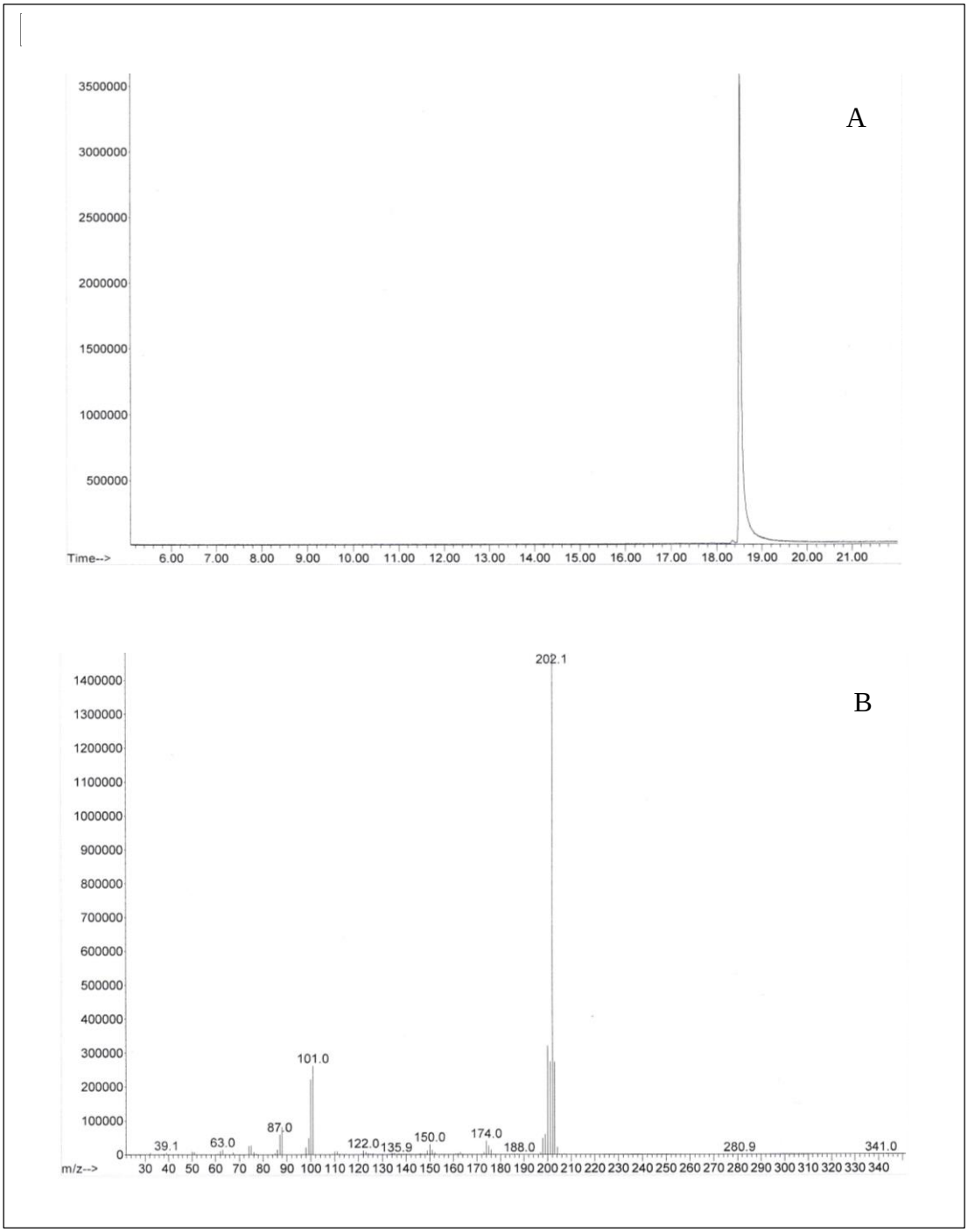

Figure 6 GC Chromatogram (A) and Mass spectrum (B) of pyrene

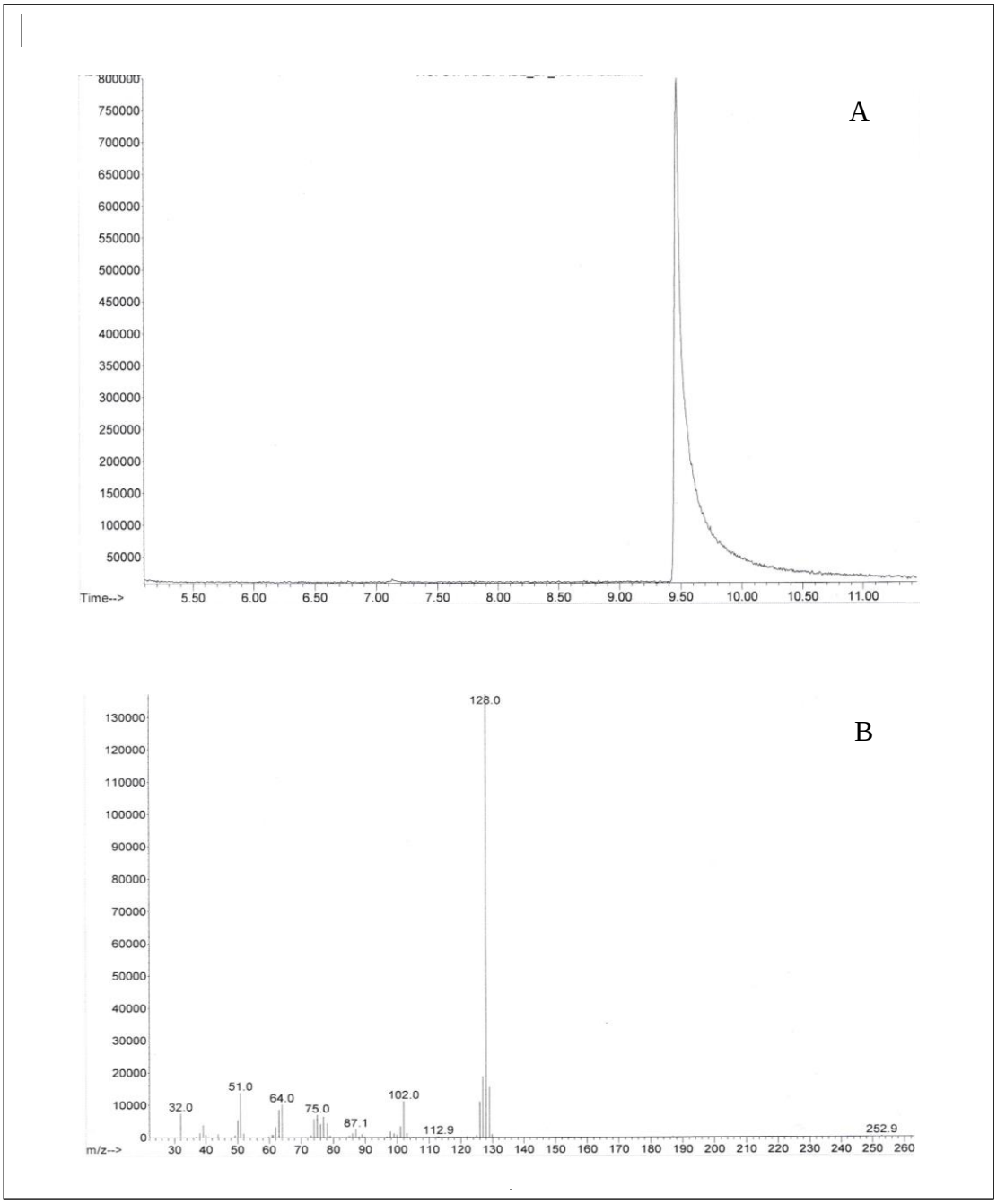

Figure 7 GC Chromatogram (A) and Mass spectrum (B) of naphthalene

4.3. Influence of Temperature on Bacterial Isolates metabolism

Biodegradation study was conducted at varying temperatures (10 ^{o}C, 25 ^{o}C and 37 ^{o}C) to elucidate PAHs metabolism potential of 7 bacterial isolates as well as 3 separate combinations of bacterial Consortia (C_2, C_4 and C_7). Circum-neutral pH (7) was held constant for each temperature range being investigated.

4.3.1. Phenanthrene degradation

Figure 8 (A-C) depicts metabolism of phenanthrene as regards the bacterial isolates studied at chosen temperatures. At mesothermic temperature (figure 8A), biodegradation activity of *Caulobacter sp* resulted in about 91% mineralization as 8.93 ppm residual PAH was detected in culture at the end of incubation time. In the media inoculated with *P. mirabilis*, concentration of PAH reduced dramatically to 5.23 ppm at day 18, indicating 94.8% biodegradation. *B. Brevis* consumed about 96.5% as levels of phenanthrene declined steeply to 3.57 ppm. Biotransformation activity of *P. borealis* yielded 96% phenanthrene decompositon with the concentration diminishing to 4.07 ppm at the end of the incubation period. *R qinshengi* and *B. fungorum* degraded over 90% of the initial phenanthrene. *Ralstonia sp* showed the least degradation at day 18.

In the media incubated at 25 ^{o}C (figure 8B), *Caulobacter sp* and *P. mirabilis* not more than 6.0 ppm residual of hydrocarbon was detected. Thus the strains separately mineralized over 93% phenanthrene. *B. fungorum* and *R. qinshengi* degraded approximately 90% and 88%

phenanthrene, respectively. The lowest biodegradation activity was however noticed in *Ralstonia sp*. Residual PAH concentration in incubation flasks at the end of day 18 fell a little below 40.00ppm, corresponding to about 60% mineralization.

When incubation was carried out at low temperatures (10 ^{o}C) as demonstrated in figure 8C, the amount of PAH biodegraded failed to exceed 60%, this holds true for all the strains investigated. The concentration of residual phenenathrene determined in cultures of *B. fungorum and R. qinshengi* were 41.47 ppm and 42.97 ppm respectively. These values represent the highest degradation recorded at 10 ^{o}C. Interestingly, residual PAH levels of 70.61 ppm was found in flasks inoculated with *P. mirabilis.* Thus the least mineralization of approximately 30% was noticed in *P. mirabilis* incubated media. Table 2 illustrates the levels of residual phenanthrene in bacterial cultures at various temperatures at the end of 18 days incubation period.

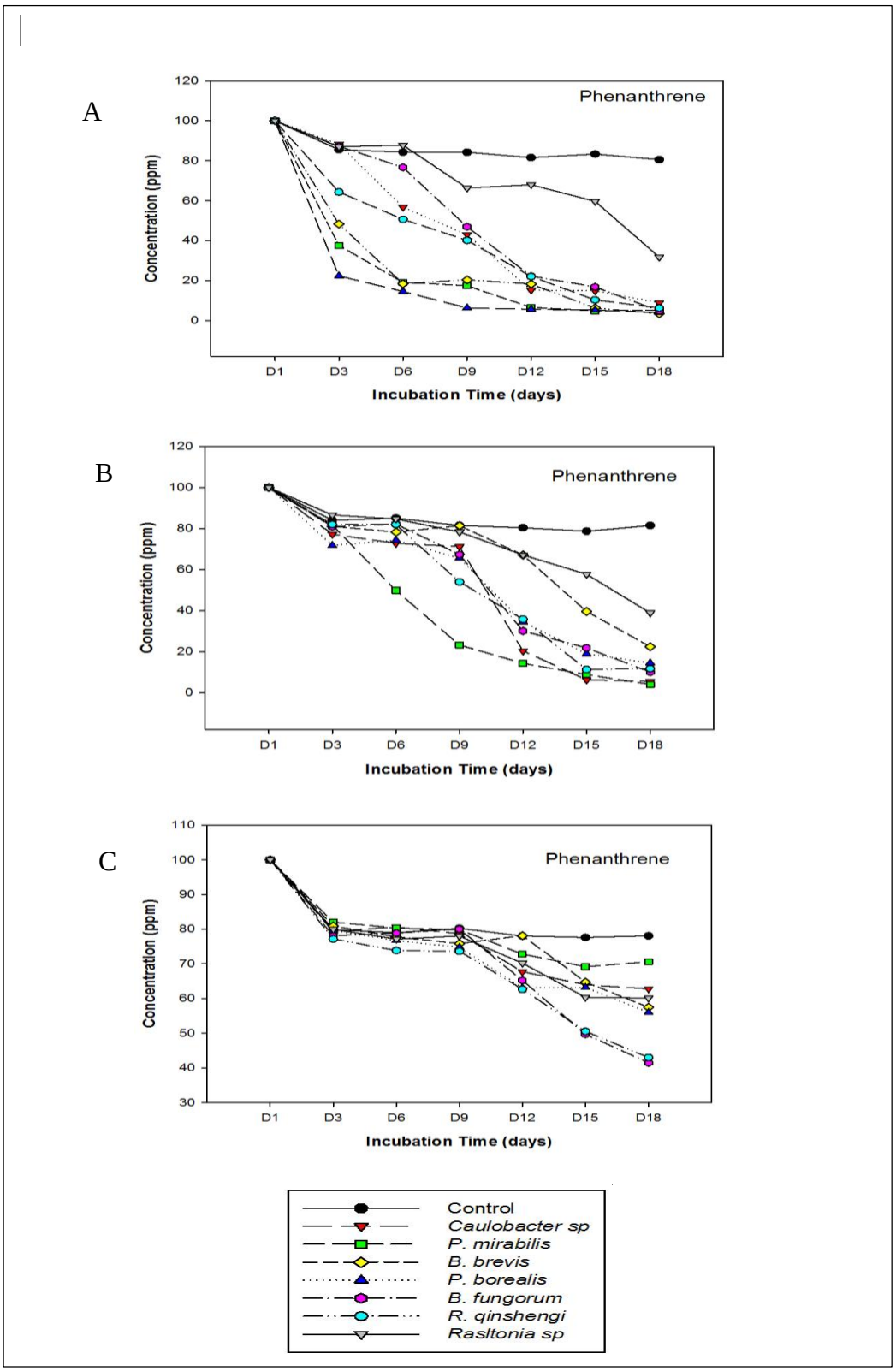

Figure 8 Phenanthrene degradation curves in bacterial isolates (A) 37ºC, (B) 25ºC and (C) 10ºC

Table 2 concentration of phenanthrene (ppm) remaining in cultures of bacterial isolates after 18 days incubation period at varying temperatures

#	Strains	10ºC		25ºC		37ºC	
		Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
1	*Caulobacter sp*	62.8	±0.33	5.42	±0.42	8.93	±0.37
2	*P. mirabilis*	70.61	±0.57	4.02	±1.01	5.23	±0.22
3	*B. brevis*	57.44	±1.12	22.41	±1.15	3.57	±0.085
4	*P. borealis*	56.00	±0.91	14.48	±1.94	4.07	±0.57
5	*B. fungorum*	41.47	±1.61	10.07	±1.62	4.84	±0.98
6	*R. qinshengi*	42.97	±0.92	11.71	±0.57	6.28	±1.32
7	*Ralstonia sp*	60.16	±0.064	38.83	±3.39	31.76	±3.38

4.3.2. Pyrene biodegradation

In this study, *B. fungorum* and *R. qinshengi* degraded the maximum amounts of pyrene. Pyrene degradation at mesothermic temperature is depicted in figure 9A. *B. fungorum* and *R. qinshengi* nearly 56% and 58% pyrene with 44.41 ppm and 41.88 ppm residual PAH in both cultures respectively. *P. borealis* and *P. miribilis* degraded approximately equal amounts of hydrocarbon. The concentration of unitized pyrene detected in flasks did not exceed 56 ppm; an equivalence of 44% PAH was metabolized by these strains. The lowest mineralization was noted in cultures of *Caulobacter sp, B. brevis* and *Ralstonia sp.* The amount of pyrene biodegraded by each of these strains was found be less than 36%.

The trajectory of pyrene metabolism at room temperature is shown in figure 9A. The pattern of PAH degradation appears similar to that of incubation carried out at mesothermic temperature (37 ^{o}C). *B. fungorum* was observed to have enhanced at 25 ^{o}C. The strains metabolism resulted in 59% biodegradation. In *R. qinshengi* inoculated cultures, 46 ppm residual pyrene was determined, thus 54% decomposition occurred. *Caulobacter sp, P. Mirabilis and B. brevis* decomposed between 36% -39%, the maximum residual pyrene recorded did not exceed 64 ppm. *Ralstonia sp* showed least mineralization potentials as evidenced by the relatively high residual PAH levels in the media.

Pyrene removal at 10 ^{o}C is presented in figure 9C.The levels of residual pyrene in bacterial cultures at day 18 was disproportionately lower than the levels detected in flasks incubated at mesothermic and room temperatures. *B. fungorum* and *R. qinshengi* degraded about 33% and 37%, pyrene respectively. Metabolic activities of the remaining strains was however

unable to produce above 30% mineralization as the minimum levels of residual pyrene in cultures was about 70.00ppm. Table 3 vividly illustrates the concentration of residual pyrene in bacterial cultures at the end of 18 days incubation time.

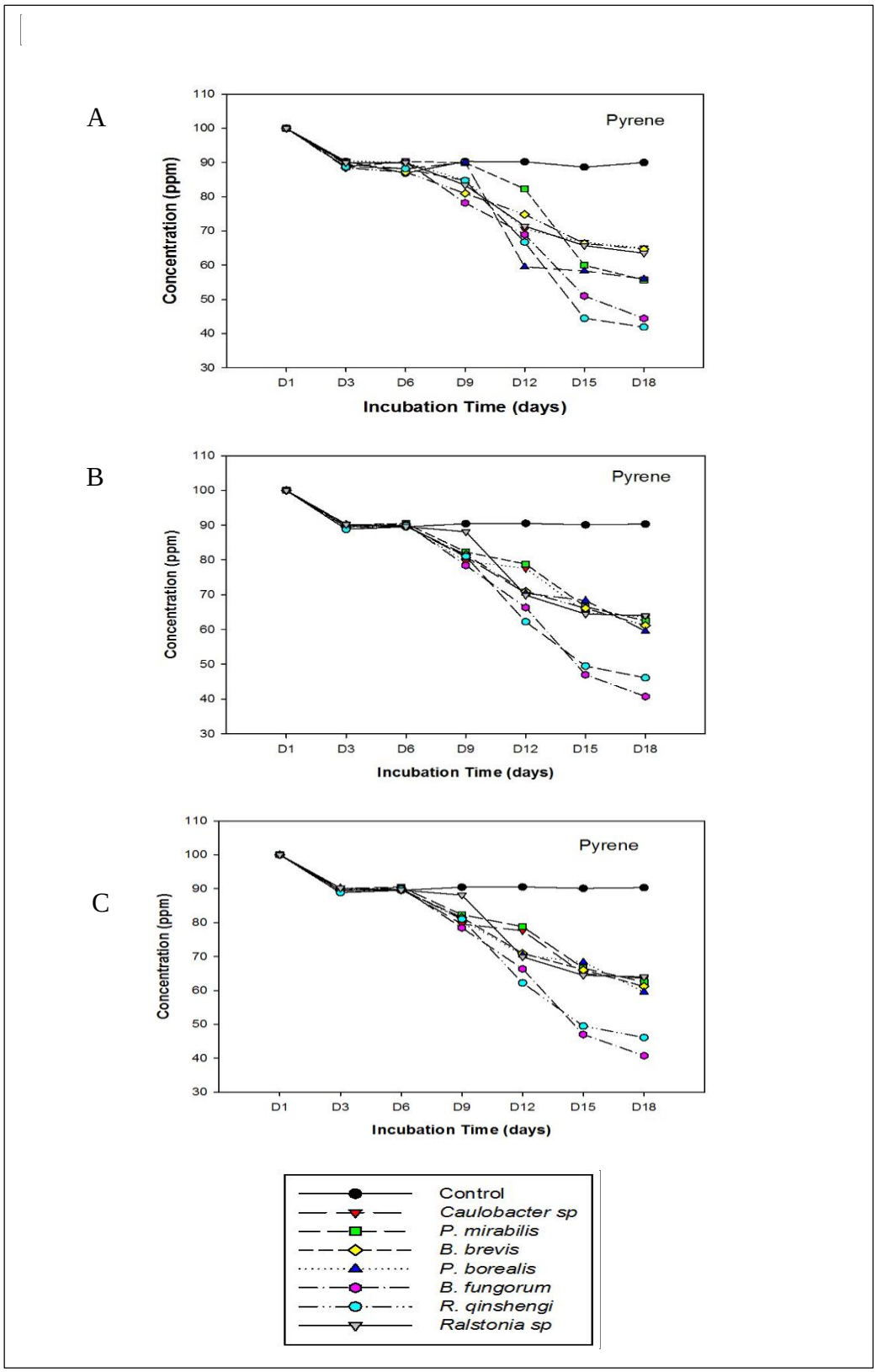

Figure 9 Pyrene degradation curves in bacterial isolates (A) 37 ^{o}C, (B) 25 ^{o}C and (C) 10 ^{o}C

Table 3 concentration pyrene (ppm) remaining in cultures of bacterial isolates after 18 days incubation period at varying temperatures

#	Strains	10ºC		25ºC		37ºC	
		Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
1	*Caulobacter sp*	78.58	±0.75	63.55	±0.74	65.01	±1.36
2	*P. mirabilis*	71.83	±0.54	62.47	±0.85	55.66	±0.67
3	*B. brevis*	77.00	±0.64	61.16	±0.23	64.69	±1.49
4	*P. borealis*	72.65	±0.81	59.50	±0.74	55.95	±0.092
5	*B. fungorum*	66.80	±0.085	40.74	±0.86	44.41	±0.81
6	*R. qinshengi*	63.11	±0.46	46.09	±0.62	41.88	±0.23
7	*Ralstonia sp*	69.87	±0.48	63.99	±2.04	63.50	±0.87

4.3.3. Naphthalene biodegradation

Figure 10 demonstrates the trajectory and pattern of bacterial naphthalene metabolism over 18 days period. After 18 days incubation under 37 ^{o}C (figure 5A), naphthalene was undetected in *B. fungorum* and *R. qinshengi* inoculated flasks. *P. mirabilis* and *P. borealis* appeared to have degraded over 95% naphthalene, leaving not less than 5ppm residual naphthalene in the media. Biodegradation activity of *Caulobacter sp* as well as *Ralstonia sp* was somewhat similar as the strains mineralization resulted in dissipation of over 93% naphthalene. *B. brevis* however recorded the lowest hydrocarbon degradation. The strain metabolized about 87% PAH.

For the strains investigated, at room temperature, each degraded over 80% naphthalene. Figure 10B illustrates bacterial metabolism pattern at 25 ^{o}C. The highest mineralization was observed in *P. mirabilis* and *R. qinshengi* inoculated flasks. The strains biodegraded not less than 94% naphthalene. Metabolic activity of *P. borealis* and *B. fungorum* yielded approximately 90% degradation as the concentration of remaining PAH at day 18 did not exceed 11 ppm. *B. brevis* remains the strain with least metabolic activity.

Figure 10C elucidates the trend in microbial biodegradation at low temperature (10^{o}C). The greatest degradation activity was found in *B. fungorum* and *R. qinshengi* inoculated cultures. These strains mineralized 61% and 64% naphthalene respectively. *P. borealis* degraded over 50% of PAH, while metabolism of naphthalene by *Caulobacter sp, B. brevis* and *Ralstonia sp* though reduced profoundly at 10^{o}C, did not fall below 30%. The maximum level of residual PAH detected was 63.84ppm in *Caulobacter sp* inoculated

cultures. Amounts of unutilized naphthalene in various bacterial cultures is summarized in Table 4.

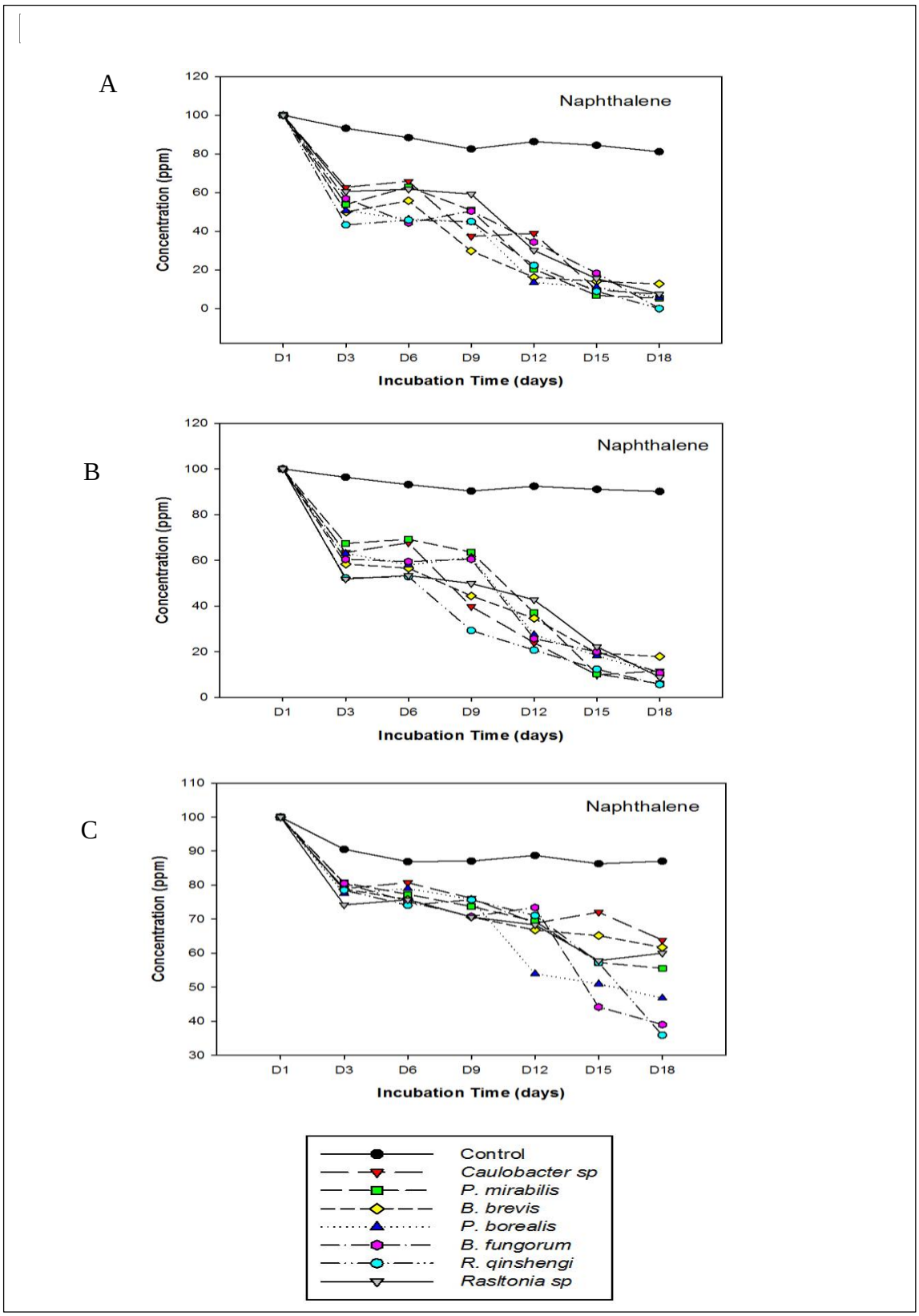

Figure 10 Naphthalene degradation curves in bacterial isolates (A) 37 ºC, (B) 25 ºC and (C) 10 ºC

Table 4 concentration of naphthalene (ppm) remaining in bacterial cultures of bacterial isolates after 18 days incubation at varying temperatures

#	Strains	10ºC		25ºC		37ºC	
		Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
1	*Caulobacter sp*	63.84	±0.28	11.57	±2.32	7.33	±1.10
2	*P. mirabilis*	55.56	±0.73	5.95	±0.37	5.4	±0.15
3	*B. brevis*	61.67	±0.64	17.84	±0.89	12.73	±0.42
4	*P. borealis*	46.78	±0.34	10.11	±0.85	5.61	±0.42
5	*B. fungorum*	39.00	±0.06	10.72	±0.86	ND	-
6	*R. qinshengi*	35.88	±0.63	5.63	±0.35	ND	-
7	*Ralstonia sp*	60.00	±1.36	8.84	±0.69	7.51	±0.30

4.4. Influence of Temperature on Consortium Metabolism

Microbial consortium potentials to metabolize PAH was investigated over temperature range of 10 ^{o}C – 37 ^{o}C. Consortium metabolism of PAHs enhanced compared to bacterial isolates degradation. At 25 ^{o}C and 37 ^{o}C, biodegradation was however several orders of magnitude greater than that of 10 ^{o}C. The sections below highlight bacterial consortium metabolism of PAHs at varying temperatures.

4.4.1. Phenanthrene degradation

Table 5 highlights residual hydrocarbon levels in flasks after incubation at various temperatures. Interestingly, phenanthrene was not detected after analysis was performed on samples inoculated with C_7. Bacterial co-metabolic activity culminated in total disappearance on the PAH at 37^{o}C (figure 11A). C_2 and C_4 consortia showed about 97% phenanthrene degradation, as only 3ppm residual PAH was detected in both cultures.

Biodegradation pattern under room temperature is shown in figure 11B. The concentration of phenanthrene declined to 2.66ppm in C_7 incubated media, corresponding to nearly 97% degradation. C_4 and C_2 mineralized approximately 96% and 95% phenanthrene, respectively. Bacterial metabolism under low temperatures decreased remarkably (figure 11C). The concentration of unutilized PAH detected in cultures of C_7 and C_4 after incubation time was 31.57ppm and 37.99ppm, representing about 68% and 62%

degradation respectively. The lowest mineralization activity was recorded in C_2 inoculated samples. The average degradation in C_7 and C_4 inoculated samples appeared somewhat higher than C_2.

Table 5 concentration of phenanthrene (ppm) remaining in consortium inoculated cultures after 18 days incubation at varying temperatures

#	Consortiums	10ºC		25ºC		37ºC	
		Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
1	**Consortium (C2)**	48.14	±0.25	4.18	±0.21	3.06	±0.078
2	**Consortium (C4)**	37.99	±0.75	4.64	±0.47	2.78	±0.057
3	**Consortium (C7)**	31.57	±1.31	2.66	±0.064	ND	-

Note;

ND = Not detected

C2 = (*P. mirabilis + P. borealis*)

C4 = (*Caulobacter sp + B. fungorum + P. mirabilis + P. borealis*)

C7 = (*Caulobacter sp + B. fungorum + P. mirabilis + P. borealis +R. quinshengi+ Ralstonia sp + B. brevis*)

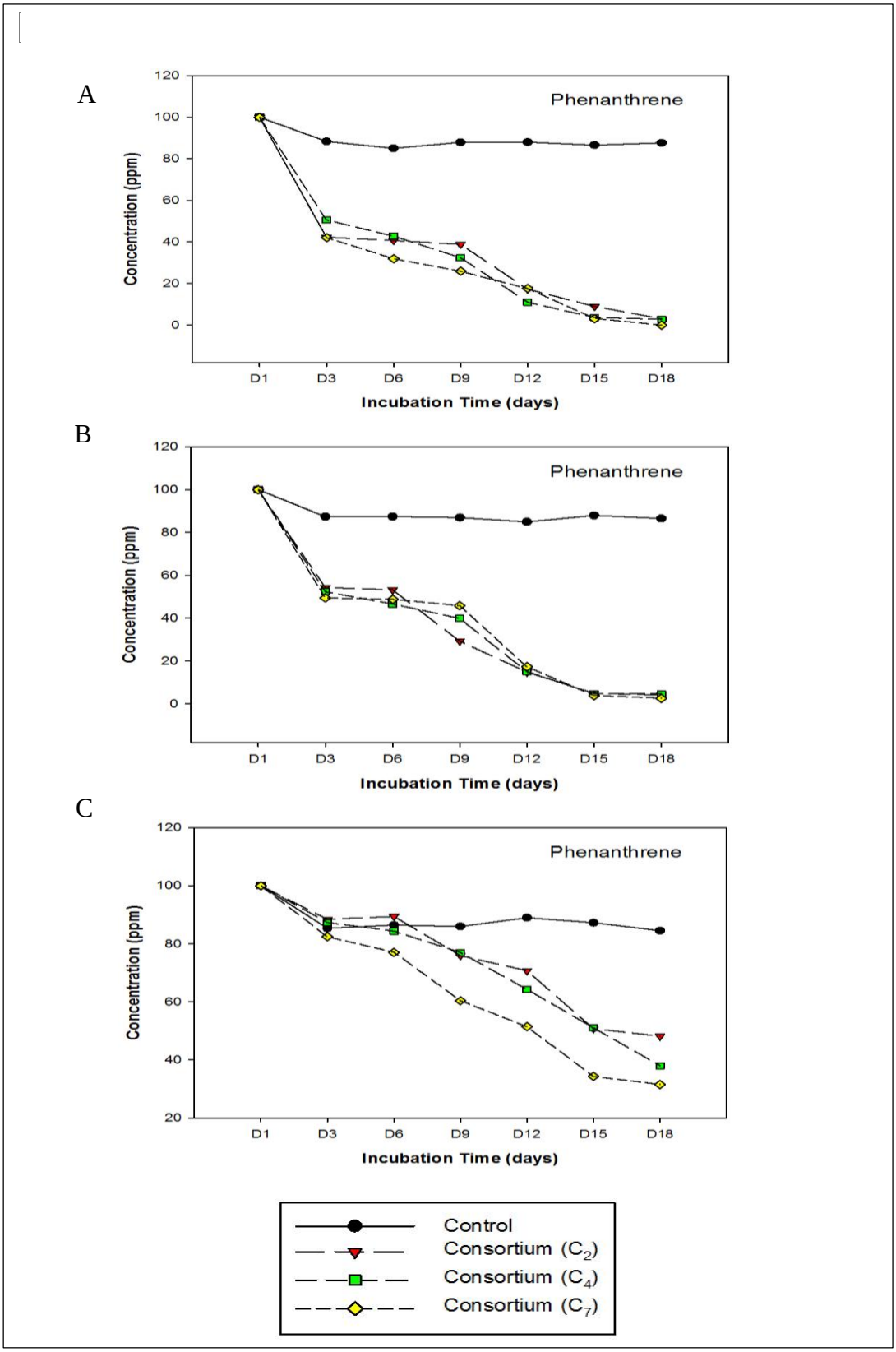

Figure 11 Phenanthrene degradation curves in consortium (A) 37ºC, (B) 25ºC and (C) 10ºC

4.4.2. Pyrene degradation

Table 6 summarizes the concentrations of remaining pyrene in samples analyzed after 18 days incubation. As visible from the biodegradation trajectory in figure 12A, C_7 metabolized 78% PAH. C_4 and C_2 biodegraded 64% and 49% pyrene, leaving residual PAH of 35.89 ppm and 51.11 ppm in respective media.

The maximum biodegradation observed at 25 ^oC (figure12B) was not more than 75% for C_7 consortium. C_2 metabolism yielded the least mineralization of 45% pyrene. The consortia investigated elicited somewhat closed levels of residual PAH in cultures at 10 ^oC. Under this temperature, 55 ppm and 56 ppm residual pyrene was detected in C_7 and C_4 media, thus not less than 45% pyrene was mineralized. Figure 12C depicts consortia metabolism at 10 ^oC. However, residual pyrene as high as 60ppm was observed in C_2 cultures.

Table 6 concentration of pyrene (ppm) remaining in cultures of consortium after 18 days varying temperatures

#	Consortiums	10°C		25°C		37°C	
		Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
1	**Consortium (C2)**	59.5	±0.75	55.14	±1.31	51.11	±1.55
2	**Consortium (C4)**	56.18	±0.2	39.99	±0.18	35.89	±1.44
3	**Consortium (C7)**	54.64	±1.25	35.03	±0.30	21.85	±1.13

Note;

C2 = (*P. mirabilis + P. borealis*)

C4 = (*Caulobacter sp + B. fungorum + P. mirabilis + P. borealis*)

C7 = (*Caulobacter sp + B. fungorum + P. mirabilis + P. borealis +R. quinshengi+ Ralstonia sp + B. brevis*)

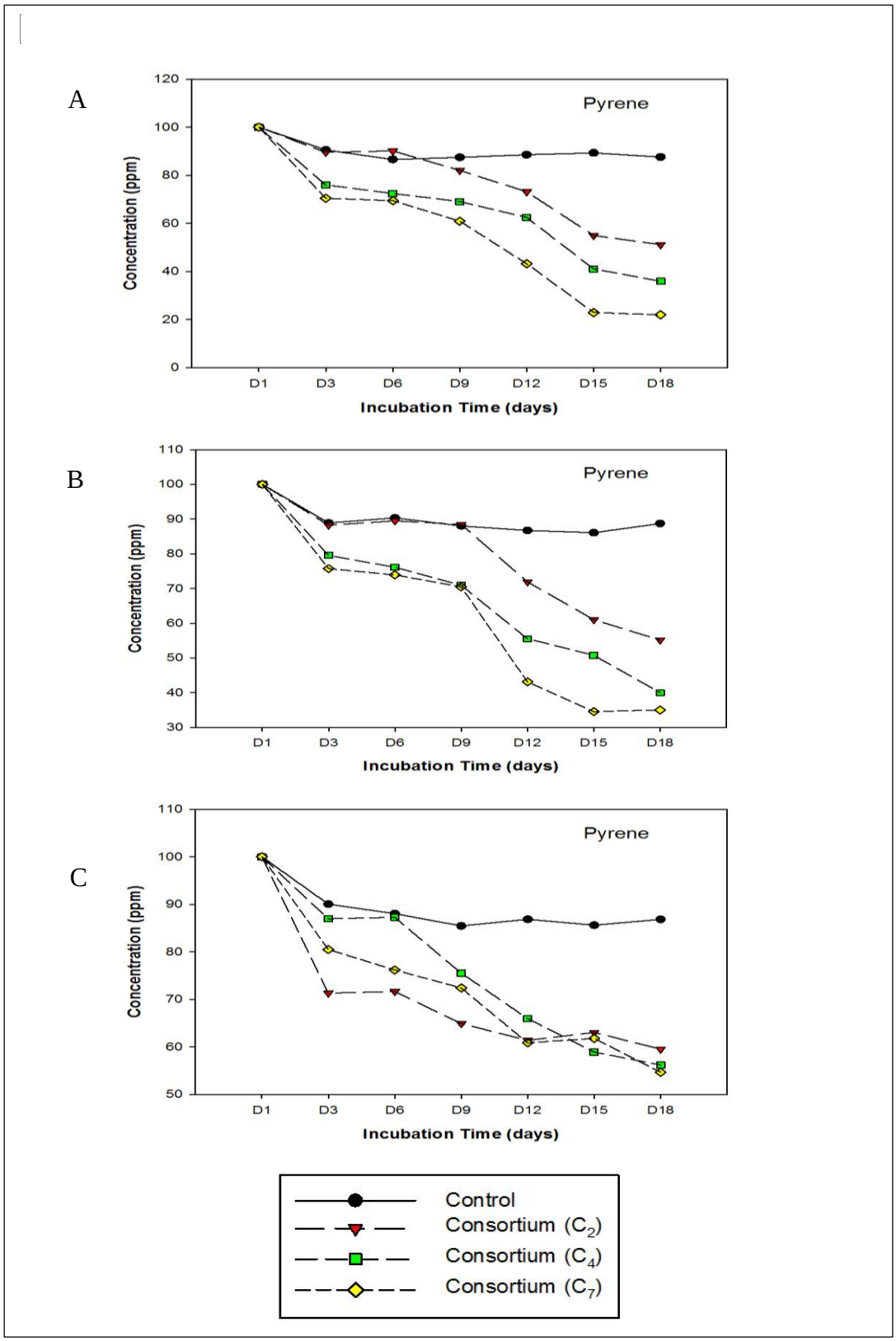

Figure 12 Pyrene degradation curves in consortium (A) 37 ºC, (B) 25 ºC and (C) 10 ºC

4.4.3. Naphthalene degradation

Bacterial consortium metabolism of naphthalene between 10 ^{o}C – 37 ^{o}C over 18 days incubation time has been demonstrated in figure 13 (A-C). As shown in table 7, at mesothermic and temperature, consortium metabolic activity resulted in absolute depletion of the naphthalene in cultures of C_2, C_7 and C_4 (13A).

Under room temperature, C_2 degraded 94.6% naphthalene with residual PAH being 5.43ppm at 18d incubation (figure 13B). There was no un-metabolized naphthalene detectable in C_4 and C_7 incubated samples. Conversely, residual naphthalene was determined in the media incubated at 10 ^{o}C (figure 13C). The average amount of naphthalene degraded fell below 65%. C_7 mineralized about 72% naphthalene. The least degradation was noticed in C_2 incubated flasks with approximately 38 ppm unutilized naphthalene.

Table 7 concentration of naphthalene (ppm) remaining in cultures of consortium after 18 days incubation at varying temperatures

#	Consortiums	10ºC		25ºC		37ºC	
		Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
1	**Consortium (C2)**	37.59	±0.70	5.43	±0.11	ND	-
2	**Consortium (C4)**	35.59	±0.70	ND	-	ND	-
3	**Consortium (C7)**	27.72	±0.85	ND	-	ND	-

Note;

ND = Not detected

C2 = (*P. mirabilis + P. borealis*)

C4 = (*Caulobacter sp + B. fungorum + P. mirabilis + P. borealis*)

C7 = (*Caulobacter sp + B. fungorum + P. mirabilis + P. borealis +R. quinshengi + Ralstonia sp + B. brevis*)

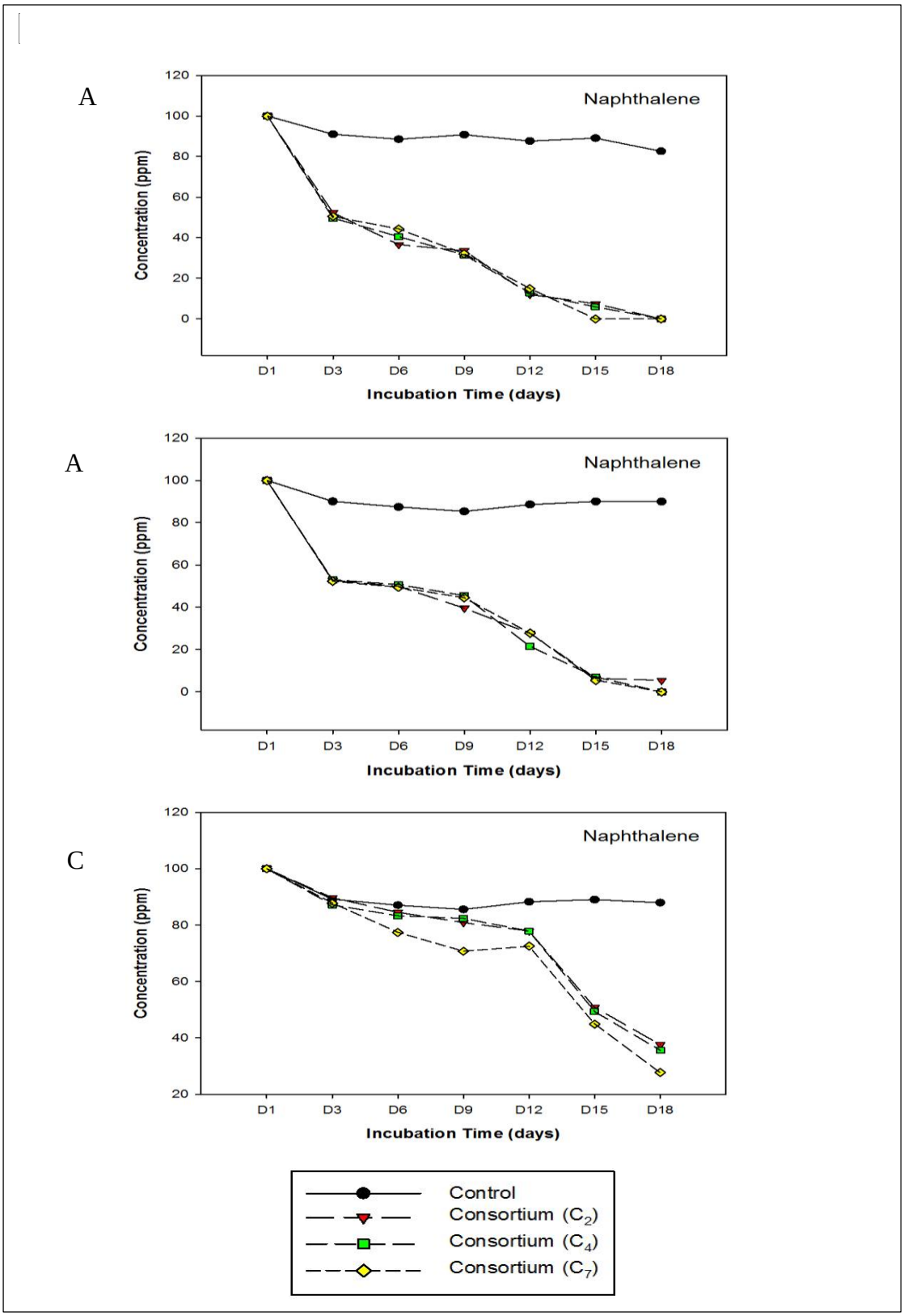

Figure 13 Naphthalene degradation curves in consortium (A) 37 ºC, (B) 25 ºC and (C) 10 ºC

4.5. Influence of pH on Bacterial Isolates Metabolism

In this study, metabolism of PAHs by bacteria under different pH ranges was variable, with maximum degradation occurring at circum-neutral pH.

4.5.1. Phenanthrene biodegradation

Biodegradation of at circum-neutral pH (7.0) is shown in figure 14A. The strain *Caulobacter sp* mineralized about 91% phenanthrene, with 8.93 ppm residual PAH remaining in samples. In the media inoculated with *P. mirabilis*, approximately 95% biodegradation transpired as the initial 100 ppm PAH diminished to 5.23 ppm at day 18. *B. brevis* and *P. borealis* metabolism was not significantly different. These strains both mineralized not less than 96% phenanthrene. *R qinshengi* and *B. fungorum* degraded over 90% of the initial phenanthrene. *Ralstonia sp* showed the least degradation value of about 68% at day 18. Table 8 elucidates the levels of residual phenanthrene in samples incubated between pH 5.0-9.0.

Decomposition of phenanthrene in acidic media showed remarkable variation compared with that of neutral environment. *P. borealis* and *B. fungorum* degraded the highest amount of PAH in acidic medium (figure 14B). The strains utilized 87% and 90% phenanthrene, leaving 13.00 ppm and 10.00 ppm, respectively. *P. mirabilis* and *R. qinshengi* metabolized nearly equal quantum of PAH, these strains activity accounted for about 80% dissipation

of phenanthrene at the end of day 18. The lowest mineralization was realized in *Ralstoina sp* inoculated media. Residual phenanthrene as high as 41.56 ppm was detectable at day 18.

Bacterial uptake and assimilation of PAH in alkaline media appeared profoundly reduced with some degree of similarity to that of biodegradation in acidic conditions (figure 14C). Interestingly, compared with acidic the medium, *Ralstonia sp* metabolic activity increased tremendously in pH 9.0. The strain degraded not less than 75% phenanthrene. *P. mirabilis* degraded nearly 81% phenanthrene with residual PAH of 19.39 ppm in media. *P. borealis* and *R. qinshengi* degraded 78% phenanthrene. The least metabolic activity was however noticed in *Caulobacter sp* incubated samples. Table 8 presents the concentrations of unutilized phenanthrene in bacterial cultures at the end of 18 days biodegradation.

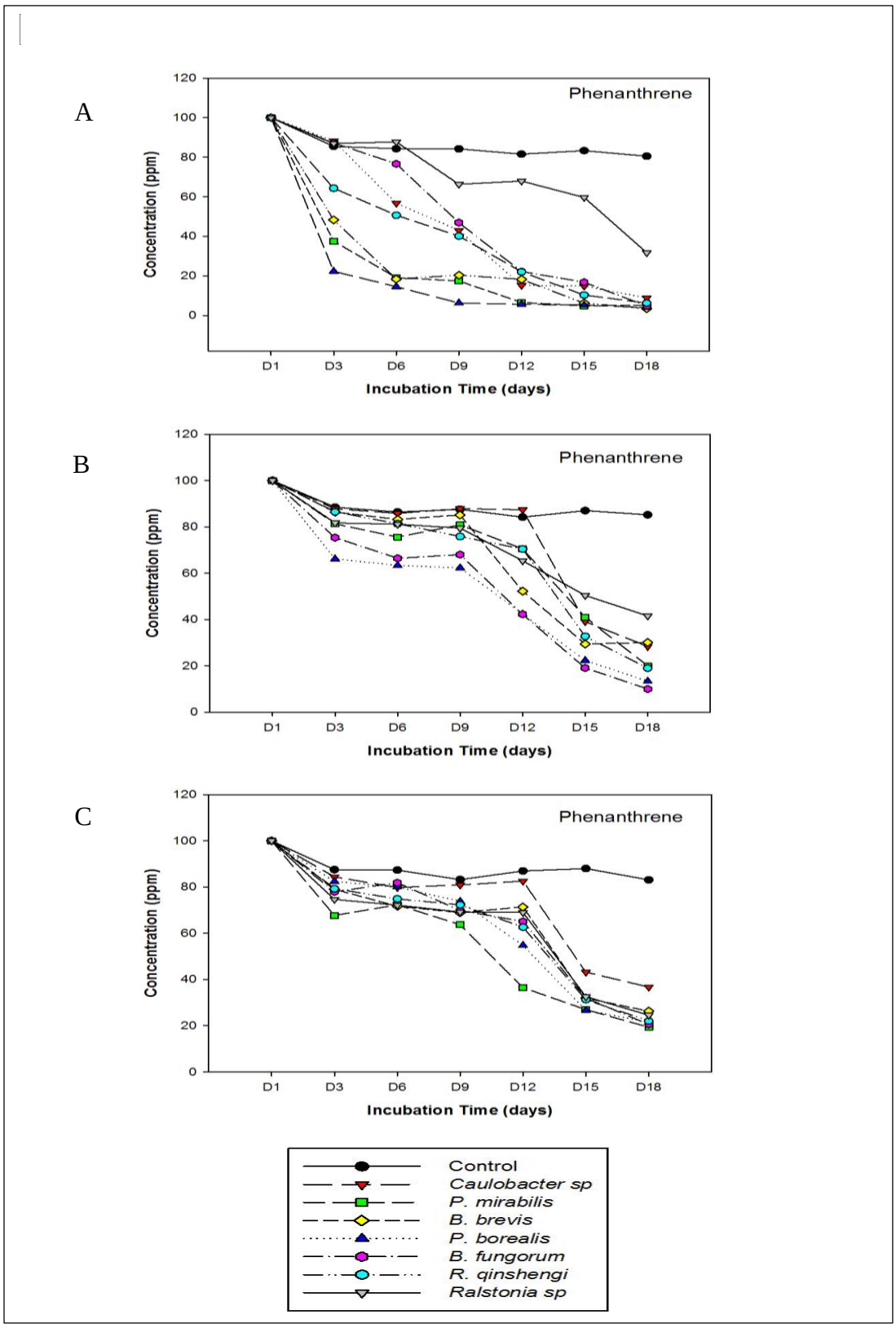

Figure 14 Phenanthrene degradation curves in bacterial isolates at pH (A) 7.0, (B) 5.0 and (C) 9.0

Table 8 concentration of phenanthrene (ppm) remaining in cultures of bacterial isolates at pH of 5.0-9.0 after 18 days incubation

#	Strains	pH 5.0		pH 9.0		pH 7.0	
		Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
1	*Caulobacter sp*	28.35	±1.84	36.69	±0.91	8.93	±0.37
2	*P. mirabilis*	19.98	±0.13	19.36	±0.66	5.23	±0.22
3	*B. brevis*	30.05	±1.38	26.26	±0.88	3.57	±0.085
4	*P. borealis*	13.38	±1.34	21.56	±0.64	4.07	±0.57
5	*B. fungorum*	10.04	±1.39	20.58	±0.66	4.84	±0.98
6	*R. qinshengi*	19.02	±0.57	22.05	±2.68	6.28	±1.32
7	*Ralstonia sp*	41.56	±0.74	24.56	±0.73	31.76	±3.38

4.5.2. Pyrene biodegradation

Table 9 sheds light on concentrations of residual detected pyrene detected in various bacterial cultures after 18 day incubation. Pyrene degradation at neutral pH is depicted in figure 15A. *B. fungorum* metabolized approximately 58% pyrene. Metabolic activity of *R. qinshengi* resulted in depletion of about 56% pyrene. In *P. borealis* and *P. miribilis* incubated inoculated media, concentrations of pyrene detected did not exceed 56ppm. The strains consumed over 40% PAH. *Caulobacter sp, B. brevis* and *Ralstonia sp* each degraded below 35% pyrene.

Under acidic conditions, *P. borealis, B. fungorum* and *R. qinshengi* metabolism yielded approximately 48%, 59% and 61% biodegradation respectively. *P. mirabilis* and *B. brevis* each degraded not less than 26% pyrene. *Caulobacter sp* metabolism was least as it mineralized only 24% PAH with about 76 ppm of remaining pyrene in incubation flasks. Figure 15B illustrates bacterial metabolism over the 18 days incubation.

Figure 15C shows biodegradation trend in bacterial isolates in alkaline environment. Pyrene degradation as low as 21% was observed in *Caulobacter sp* inoculated samples. Contrarily, the metabolism of *P. borealis* and *B. fungorum* enhanced compared to neutral pH. The strains degraded 56% and 62% pyrene with 44.20 ppm and 37.80 ppm remaining in cultures, respectively.

Table 9 concentration of pyrene (ppm) remaining in cultures of bacterial isolates after 18 days incubation under varying pH

#	Strains	pH 5.0		pH 9.0		pH 7.0	
		Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
1	*Caulobacter sp*	74.89	±0.2	78.74	±0.98	65.01	±1.36
2	*P. mirabilis*	74.1	±0.49	70.89	±0.028	55.66	±0.67
3	*B. brevis*	74.05	±0.86	71.81	±0.40	64.69	±1.49
4	*P. borealis*	51.65	±1.37	44.2	±0.61	55.95	±0.092
5	*B. fungorum*	40.73	±0.86	37.8	±0.88	44.41	±0.81
6	*R. qinshengi*	39.16	±1.20	71.34	±1.53	41.88	±0.23
7	*Ralstonia sp*	71.93	±0.13	73.72	±0.98	63.50	±0.87

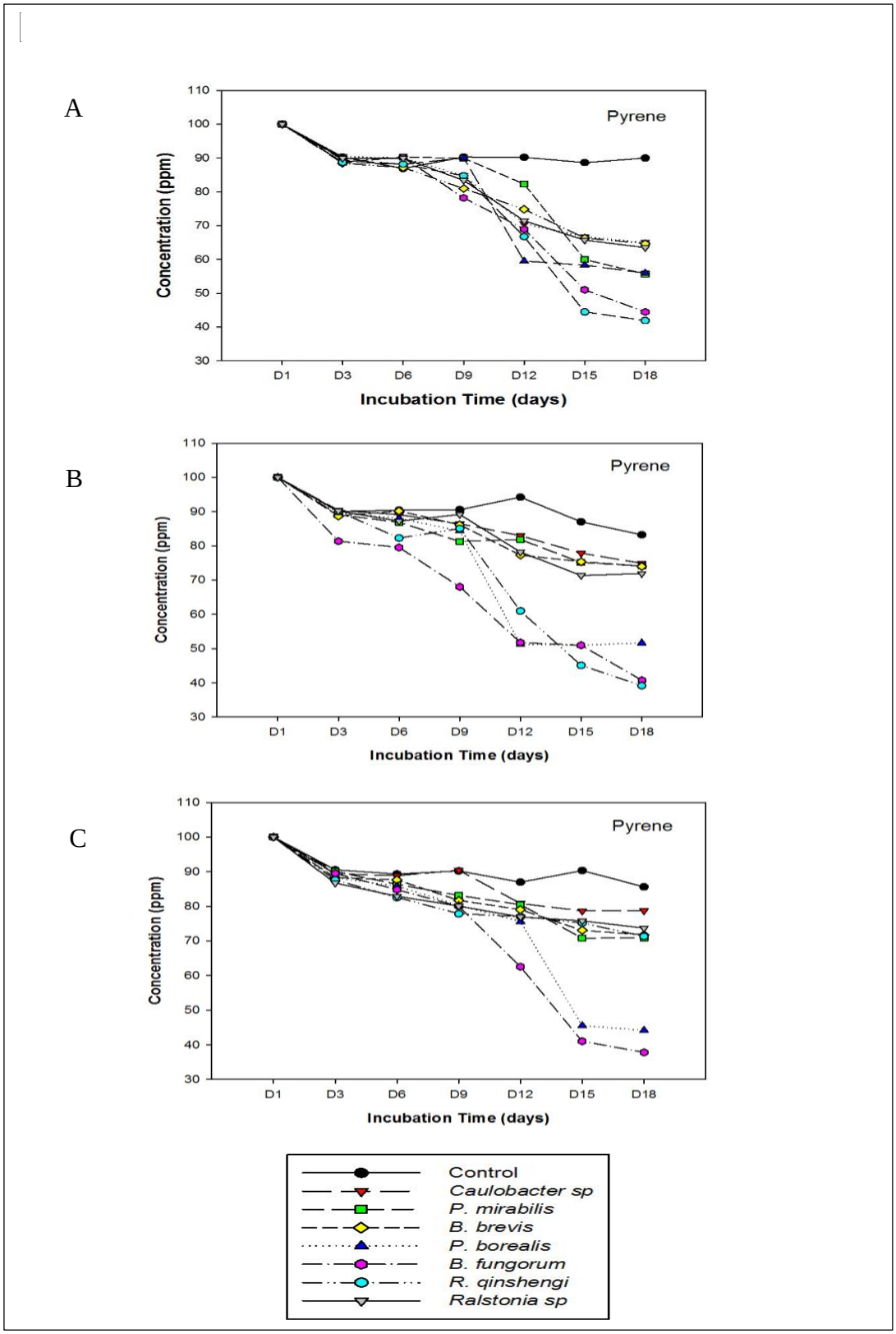

Figure 15 Pyrene degradation curves in bacterial isolates at pH (A) 7.0, (B) 5.0 and (C) 9.0

4.5.3. Naphthalene biodegradation

Figure 16 illustrates the trajectory of bacterial degradation of naphthalene. Following 18 days incubation under circum-neutral pH (figure 16A), there was no naphthalene r in media incubated with *B. fungorum* and *R. qinshengi*. *P. mirabilis* and *P. borealis* degraded over 95% naphthalene. *Caulobacter sp* and *Ralstonia sp* utilized not less than 93% naphthalene. The least degradation of about 87% was confirmed in *B. brevis* inoculated samples.

Biodegradation of naphthalene under acidic conditions is depicted by figure 16B. The maximum degradation of about 93% was recorded in *R. qinshengi* inoculated flasks. Residual PAH determined in *B. fungorum* cultures was approximately 11.00 ppm, corresponding to 89% mineralization. *P. mirabilis* and *P. borealis* utilized closely similar amounts of PAH. The strains biodegraded a little above 75% naphthalene. Unutilized PAH determined in *B. brevis* incubated media was 33.31 ppm, about 67% naphthalene was lost.

Naphthalene degradation in alkaline media can be seen in figure 16C. The lowest residual PAH (4.5 ppm) was determined in *R. qinshengi* incubated media, corresponding to 95.5% biodegradation. *P. mirabilis* degraded the least amount of PAH. *P. borealis, Caulobacter sp* and *B. fungorum* metabolized above 80% naphthalene. Table 10 shows levels of residual PAH detected in bacterial cultures at day 18.

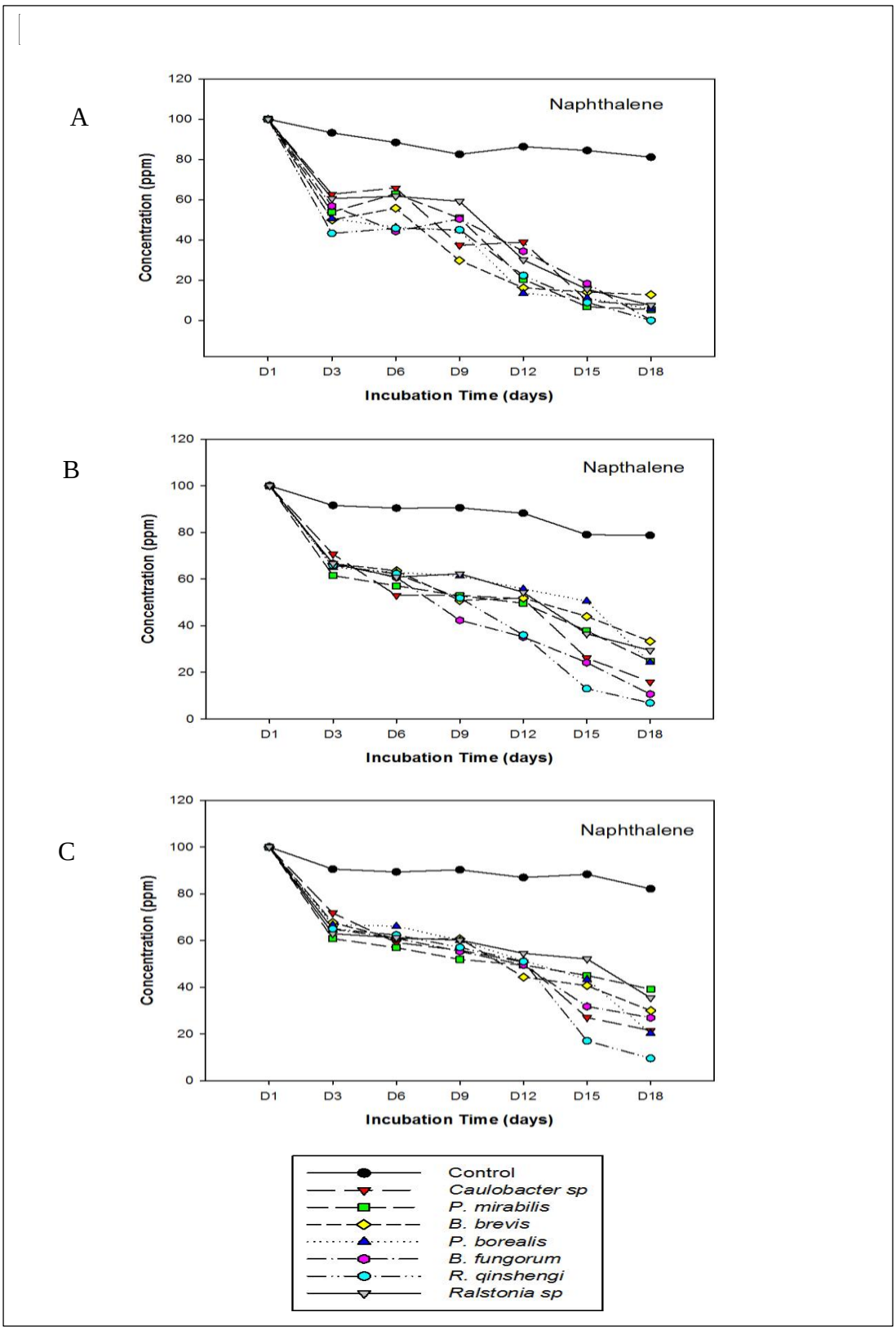

Figure 16 Naphthalene degradation curves in bacterial isolates at pH (A) 7.0, (B) 5.0 and (C) 9.0

Table 10 concentration of naphthalene (ppm) remaining cultures of bacterial isolates after 18 days incubation under varying pH ranges

#	Strains	pH 5.0		pH 9.0		pH 7.0	
		Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
1	*Caulobacter sp*	15.91	±1.12	21.44	±1.28	7.33	±1.10
2	*P. mirabilis*	24.81	±0.33	39.13	±1.27	5.40	±0.15
3	*B. brevis*	33.31	±1.42	29.99	±1.47	12.73	±0.42
4	*P. borealis*	24.24	±1.13	20.27	±1.05	5.61	±0.42
5	*B. fungorum*	10.68	±0.93	26.93	±1.97	ND	-
6	*R. qinshengi*	6.90	±0.21	9.52	±0.73	ND	-
7	*Ralstonia sp*	29.43	±1.41	35.44	±1.43	7.51	±0.30

Note;

ND = Not detected

4.6. Effects of pH on Bacterial Consortium Metabolism

PAHs mineralization was noticed have elevated by deploying microbial consortium compared with bacterial Isolates. Bacterial metabolism of specific PAHs at pH range of 5.0 - 9.0 is elucidated below.

4.6.1. Phenanthrene biodegradation

Residual hydrocarbon concentrations at day 18 is summarized in table 11. Mineralization activity of C_2 and C_4 consortia culminated in nearly 97% disappearance of phenanthrene. In the media incubated using C_7, phenanthrene was not detected. The pattern of bacterial metabolism is shown in figure 17A.

Biodegradation of phenanthrene by C_7 and C_4 in acidic medium was not significantly different as the consortia degraded a little above 80% of the initial PAH. The consortium C_2 metabolized the lowest amount of phenanthrene. Unutilized PAH detected in the cultures of C_2 was about 29.00 ppm, representing 71% biodegradation. Figure 17B sheds light on the trend in phenanthrene mineralization over the entire incubation period.

Consortia metabolic activity under alkaline condition is illustrated in Figure 17C. The consortia C_4 and C_7 degraded 80% and 79% phenanthrene respectively. The consortium C_2 mineralized the least amount of hydrocarbon. Residual PAH levels as high as 32 ppm was

found in cultures of C_2. Figure 17C illustrates the strains metabolic activity over varying incubation times in alkaline medium.

Table 11 concentration of phenanthrene (ppm) remaining in cultures of consortium after 18 days incubation under pH range of 5.0-9.0

#	Consortiums	pH 5.0		pH 9.0		pH 7.0	
		Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
1	**Consortium (C2)**	28.51	±0.65	31.96	±1.29	3.06	±0.078
2	**Consortium (C4)**	20.94	±0.72	19.95	±0.11	2.78	±0.057
3	**Consortium (C7)**	20.48	±1.49	18.52	±1.69	ND	-

Note;

C2 = (*P. mirabilis + P. borealis*)

C4 = (*Caulobacter sp + B. fungorum + P. mirabilis + P. borealis*)

C7 = (*Caulobacter sp + B. fungorum + P. mirabilis + P. borealis +R. quinshengi + Ralstonia sp + B. brevis*)

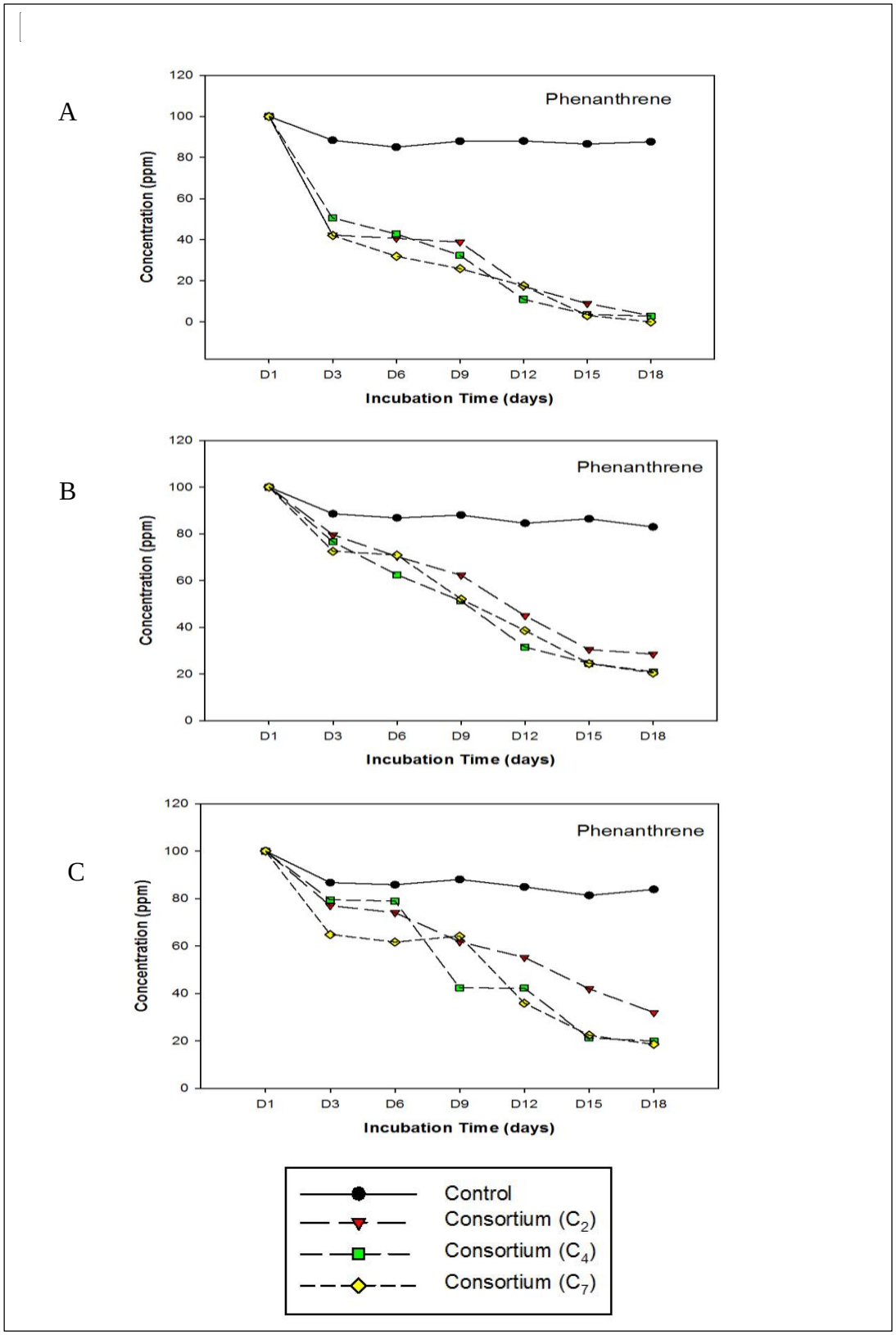

Figure 17 Phenanthrene degradation curves in consortium at pH (A) 7.0, (B) 5.0 and (C) 9.0

4.6.2. Pyrene biodegradation

Pyrene concentration in cultures of consortium after 18day biodegradation is presented in table 12. The consortium C_7 mineralized a little above 80% pyrene. Residual pyrene detected in cultures of C_2 exceeded 50.00 ppm. The consortium therefore degraded below 50% PAH. Pyrene mineralization trend in neutral pH is illustrated in figure 18A. C_4 biodegraded about 60% pyrene, resulting in less than 40.00 ppm un-metabolized PAH remaining in incubation flasks.

Figure 18B explicitly depicts pyrene degradation in acidic cultures. The metabolism of pyrene by C_2 was observed to have improved in acidic environment. The consortium degraded about 60% PAH. Maximum of 75% biodegradation transpired in C_7 inoculated media. The bacterial consortium C_4 mineralized a little beyond 70% of the PAH.

Similarly, C_2 metabolism enhanced in alkaline medium, producing over 50% mineralization. The consortia C_4 and C_7 degraded less than 60% pyrene. C_2 utilized the lowest amount of pyrene while C_7 degraded the maximum, un-metabolized pyrene in both cultures stood at 43 ppm and 32 ppm, respectively. Biodegradation under alkaline conditions is shown in figure 18C.

Table 12 concentration of pyrene (ppm) remaining in cultures of consortium after 18 days incubation at pH range 5.0-9.0

#	Consortiums	pH 5.0		pH 9.0		pH 7.0	
		Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
1	**Consortium (C2)**	40.23	±1.10	42.83	±0.69	51.11	±1.55
2	**Consortium (C4)**	31.66	±2.18	37.77	±0.35	35.89	±1.44
3	**Consortium (C7)**	25.16	±0.54	32.21	±1.28	21.85	±1.13

Note;

C2 = (*P. mirabilis + P. borealis*)

C4 = (*Caulobacter sp + B. fungorum + P. mirabilis + P. borealis*)

C7 = (*Caulobacter sp + B. fungorum + P. mirabilis + P. borealis +R. quinshengi + Ralstonia sp + B. brevis*)

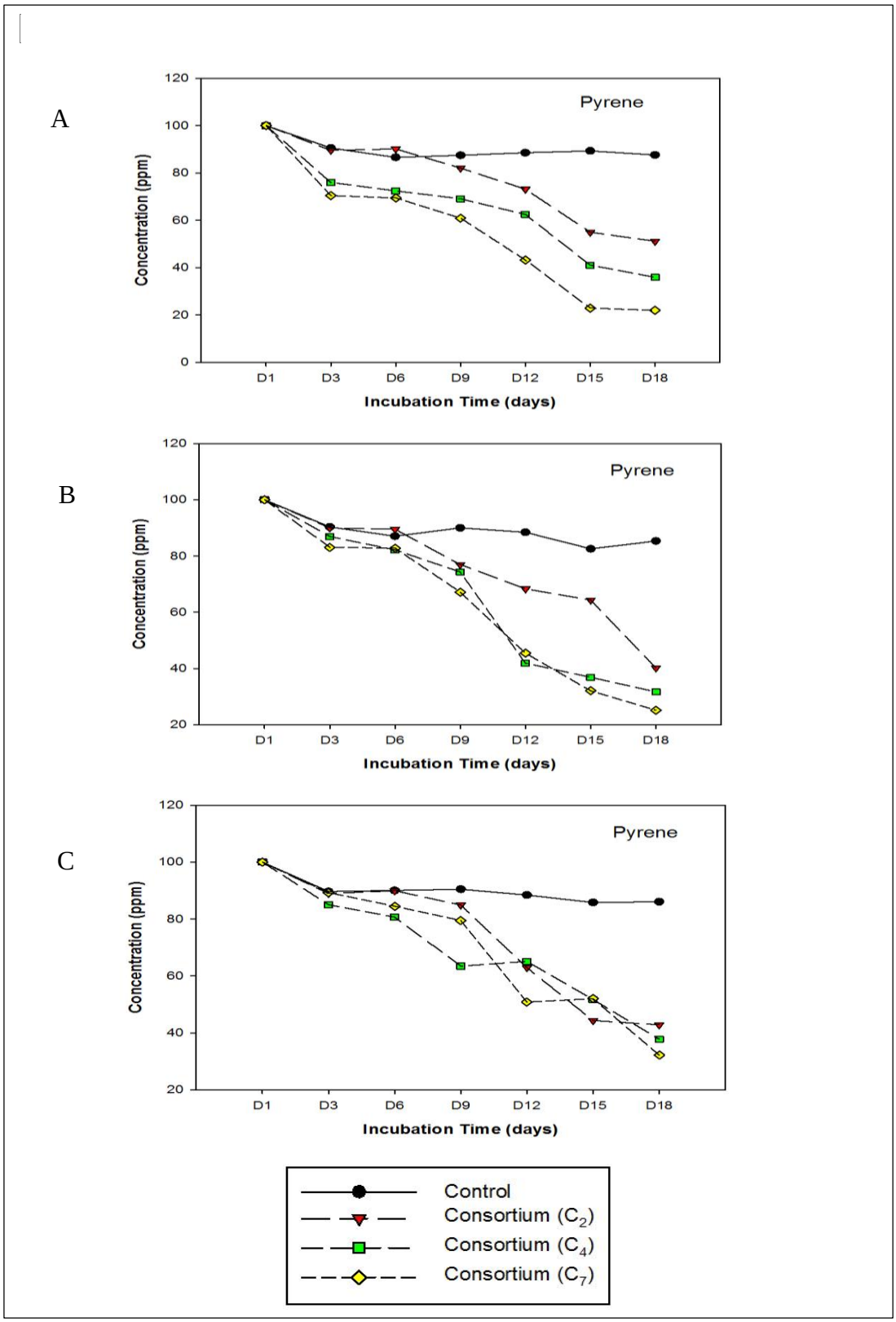

Figure 18 Pyrene degradation curves in consortium at pH (A) 7.0, (B) 5.0 and (C) 9.0

4.6.3. Naphthalene biodegradation

Table 13 shows the concentrations of residual naphthalene at the end of day 18 biodegradation. Naphthalene was undetected in consortium inoculated samples incubated at pH 7.0 (figure 19A). Bacterial PAH metabolism in acidic media is depicted in figure 19B. The consortium C_7 metabolized the maximum of approximately 91% naphthalene with 9.27ppm remaining in cultures. About 87% degradation was reckoned in C_4 incubated samples. The minimum mineralization was found in C_2 inoculated cultures as less than 80% naphthalene was utilized. Residual naphthalene in cultures of all consortia investigated in alakaline medium fell below 20.00ppm (figure 19C). Thus the Consortia metabolized not less than 80% naphthalene. Paradoxically, C_2 degraded more naphthalene (83%) than C_4 (81%).

Table 13 concentration of naphthalene (ppm) remaining in cultures of consortium after 18 days incubation under pH range of 5.0 – 9.0

#	Consortiums	pH 5.0		pH 9.0		pH 7.0	
		Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
1	**Consortium (C2)**	23.05	±1.00	16.84	±0.71	ND	-
2	**Consortium (C4)**	13.27	±0.23	18.8	±0.76	ND	-
3	**Consortium (C7)**	9.27	±1.34	16.05	±0.72	ND	-

Note;

ND = Not detected

C2 = (*P. mirabilis + P. borealis*)

C4 = (*Caulobacter sp + B. fungorum + P. mirabilis + P. borealis*)

C7 = (*Caulobacter sp + B. fungorum + P. mirabilis + P. borealis +R. quinshengi + Ralstonia sp + B. brevis*)

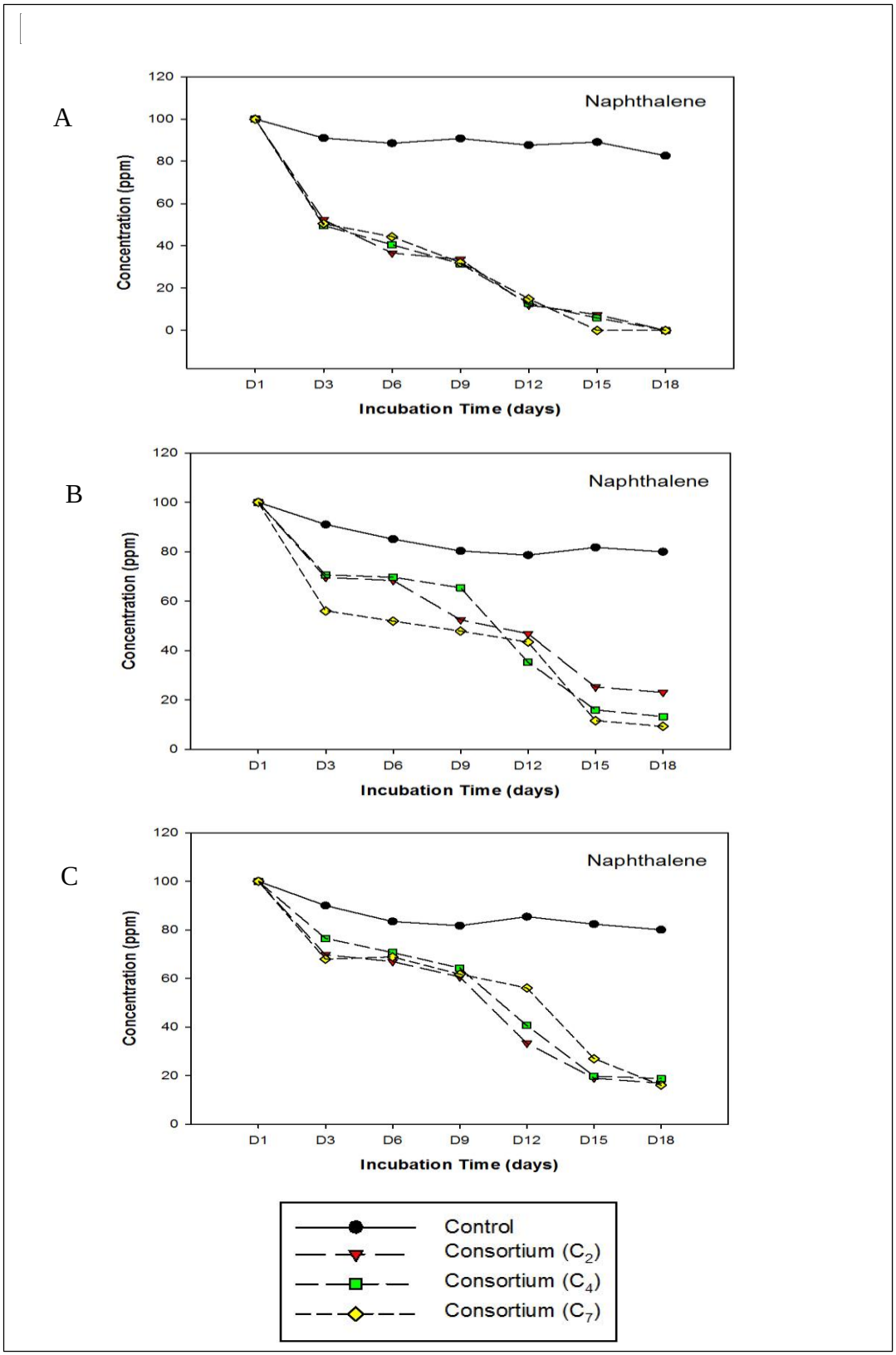

Figure 19 Naphthalene degradation curves in consortium at pH (A) 7.0, (B) 5.0 and (C) 9.0

CHAPTER 5

DISCUSSION

5.1. Biodegradation of PAHs by Isolates at varying temperatures

Biodegradation study was conducted at varying temperatures (10 ^{o}C, 25 ^{o}C and 37 ^{o}C) to elucidate PAHs removal by bacterial isolates as well as 3 consortium (C_2, C_4 and C_7). The slight dissipation of PAHs in abiotic control samples is presumably the consequence of PAHs deposition on the walls of incubation flask as water evaporates.

5.1.1. Phenanthrene biodegradation

At mesothermic temperature (figure 8A), *Caulobacter sp* removed about 91.1% phenanthrene in 18 days. The strains metabolic activity is in line with Dalal et al. [75] findings that confirmed the active participation of a certain *Caulobacter sp* in biodegradation and cleaning of crude oil contaminated sites. *P. mirabilis*, concentration of degraded 94.8% of the initial 100 ppm phenanthrene. The genera *Proteus* are able to degrade hydrocarbons [44]. *Proteus mirabilis* activity in degradation of industrial dyes is however well documented [64][65]. *B. Brevis* consumed about 96.5% as levels of phenanthrene declined steeply to 3.57 ppm. Like the genera *Proteus*, members of

Brevibacillus genera especially, *B. laterosporus* are recognized as degraders of textile dyes not PAHs [33][72][73]. Biotransformation activity of *P. borealis* yielded 96% phenanthrene degradation. The findings of Launen et al. [66] revealed PAHs metabolism pattern in *Paenibacillus* genera in a community of microorganisms. *R qinshengi* and *B. fungorum* degraded over 90% of the initial phenanthrene. This reiterates the report by de Carvalho and da Fonseca [52] and Larkin et al. [82] that *Rhodococcus sp* possesses the metabolic potential to degrade wide range of recalcitrant hydrocarbons. *Ralstonia sp* showed the least degradation at day 18. Strains belonging to *Ralstonia* genera are known to effectively assimilate and metabolized monoaromatics at 42 °C [81].

In the media incubated at 25 ^{o}C (figure 8B), *Caulobacter sp* and *P. mirabilis* decomposed over 93% phenanthrene. *B. fungorum* and *R. qinshengi* degraded approximately 90% and 88% phenanthrene, respectively. The activity of a certain *Burkholderia fungorum* in transformation of phenanthrene at 27 ^{o}C [71]. Pedetta et al.[149] reported 75 – 100% phenanthrene degradation at 25 ^{o}C deploying *Pseudomonas sp and Sphingomonas sp.* The lowest biodegradation activity (60%) was noticed in *Ralstonia sp* incubation flasks at the end of day 18. Deng et al. [150] inferred that *Achromobacter sp.* HZ01 utilized about 51% phenanthrene at 28 ^{o}C.

When incubation was carried out at low temperatures (10 ^{o}C), as demonstrated in figure 8C, the amount of PAH biodegraded failed to exceed 60%, this holds true for all the strains investigated. However, *B. fungorum and R. qinshengi* mineralized 59% and 57% respectively. These values represent the highest degradation recorded at 10 ^{o}C. Surprisingly, *P. mirabilis* degraded the lowest amount (30%) of initial 100 ppm phenanthrene.

5.1.2. Pyrene biodegradation

Pyrene comprising four fused benzene rings is notoriously persistent in the environment owing to its relatively low solubility and bioavailability, few bacteria are able to completely mineralize the hydrocarbon. Nevertheless, pyrene biodegradation mediated by *Rhodococcus* genera in contaminated environments has been reported [85][88]. In this study, *B. fungorum* and *R. qinshengi* degraded the maximum amounts of pyrene. Pyrene degradation at mesothermic temperature is depicted in figure 9A. *B. fungorum* and *R. qinshengi* nearly 56% and 52% pyrene. *Burkholderia cepacia* VUN 10,003 degraded over 70% pyrene after 5 days incubation [151]. *Mycobacterium sp*. KR2 was also found to have mineralized close to 60% of pyrene (at 500 ppm initial concentration) in 8 days [152]. In a related study, Singh et al. [153] detected 60% maximum degradation at 37 ^{o}C. *P. borealis* and *P. miribilis* degraded approximately equal amounts of hydrocarbon (figure 9A). The strains metabolized 44% of the initial 100 ppm pyrene. The lowest mineralization was noted in cultures of *Caulobacter sp, B. brevis* and *Ralstonia sp* as the amount of pyrene biodegraded by each strain was found be less than 36%. Being a HMW PAH, low degradation rate of pyrene is not surprising. Xia et al. [154] found that *Pseudomonas sp.* WJ6 could only remove 19.5% pyrene in 20 days.

There is slight variations in the strains metabolic activity at mesothermic temperature (figure 9A). For example, the performance of *B. fungorum* enhanced at 25 ^{o}C. The strains' metabolism was highest, yielding in 59% biodegradation. *Caulobacter sp, B. brevis* and *Ralstonia sp* showed least mineralization potentials. Rehmann et al. [155] reported that *Mycobacterium* strain KR2 could degrade 60% pyrene (50ppm initial concentration) at 20

^{o}C in 8 days. The initial concentration of pyrene probably inhibited complete biodegradation [156]. For instance, at initial concentration of 40 ppm, *Mycobacterium sp*. JS19b1 degraded 100% pyrene in 14 days [157].

There was a significant shift in bacterial potential to degrade phenanthrene at 10 ^{o}C (figure 9C).The levels of residual pyrene in bacterial cultures at day 18 was disproportionately lower than the levels detected in flasks incubated at mesothermic and room temperatures. The extreme low temperature though did not halt bacterial metabolism might have inhibited biodegradation coupled with low bacterial growth. Surprisingly, *B. fungorum* and *R. qinshengi* degraded about 33% and 37%, pyrene respectively. Metabolic activity of the remaining strains was however unable to produce above 30% mineralization. It is known that optimum pyrene degradation could occur above room temperatures [158][159].

5.1.3. Naphthalene biodegradation

Naphthalene being the simplest PAH is known to possess appreciably high solubility and bioavailability relative to the other PAHs with 3-5 fused benzene rings. At 18 days incubation under 37 ^{o}C (figure 10A), naphthalene was undetected in *B. fungorum* and *R. qinshengi* inoculated flasks. The possible conclusion is that 100% biodegradation transpired. Low molecular weight hydrocarbon metabolism mediated by members of the genus *Burkholderia* and *Rhodococcus* is evident in previous research [68][83]. Complete degradation of naphthalene using *Geobacillus* strain was realized in 72 hours [160]. *P. mirabilis* and *P. borealis* appeared to have degraded over 95% naphthalene.

Biodegradation activity of *Caulobacter sp* as well as *Ralstonia sp* was somewhat similar as the strains mineralization resulted in dissipation of over 93% naphthalene. *Ralstonia sp* U2 is reported to utilize methylated naphthalene as substrate via its naphthalene dioxygenase enzyme [161]. *B. brevis* however decomposed the lowest, as 87% naphthalene was dissipated.

At room temperature, bacterial metabolism of naphthalene did not diminish significantly. For the strains investigated, each degraded over 80% naphthalene (figure 10B). Interestingly, the highest mineralization was observed in *P. mirabilis* and *R. qinshengi* inoculated flasks. The strains biodegraded not less than 94% naphthalene. Metabolic activity of *P. borealis* and *B. fungorum* yielded approximately 90% degradation. Successful mineralization of naphthalene by *B. fungorum* strains at near room temperatures has been emphasized [71]. *Bacillus sp.* SBER3 could consume 75.1% naphthalene at 27 ^{o}C [162]. Compared with biodegradation under mesothermic conditions, *B. brevis* remains the strain with limited metabolic activity.

Bacterial PAH metabolism at 10 ^{o}C dropped by several orders of magnitude. Figure 10C elucidates the trend in microbial biodegradation at low temperature. The greatest degradation activity however, was found in *B. fungorum* and *R. qinshengi* inoculated cultures. These strains mineralized 61% and 64% naphthalene respectively. At temperatures ranging between 4 – 37 ^{o}C, strains in *Rhodococcus* genera are able to maintain their metabolic activity and significantly degrade hydrocarbons [83]. *P. borealis* degraded over 50% of PAH, while metabolism of naphthalene by *Caulobacter sp*, *B. brevis* and *Ralstonia sp* though reduced profoundly at 10 ^{o}C, did not fall below 30%.

5.2. PAHs degradation by Consortium at varying temperatures

Co-metabolism of hydrocarbons is known to occur in consortium of bacteria acting together to degrade hydrocarbon pollutants [59]. Consortium metabolism of PAHs enhanced compared to bacterial isolates. At 25 ^{o}C and 37 ^{o}C, biodegradation was however several orders of magnitude greater than that of 10 ^{o}C. Deploying consortium for bioremediation is found to be effective due to the diverse nature of microbial community [59]. Utilizing microbial consortia with strains of varied metabolic activities, Alisi et al. [60] and Li et al. [61] realized high rate of PAHs biodegradation.

5.2.1. Phenanthrene degradation

Phenanthrene was not detected in flasks inoculated with C_7. Bacterial co-metabolic activity culminated in 100% disappearance of the PAH at 37 ^{o}C (figure 11A). Paradoxically, metabolism of PAH by C_2 and C_4 consortia was not significantly different. About 97% of the initial 100 ppm PAH was degraded. Bacterial consortium could mineralize up to 99% phenanthrene [163]. Biodegradation pattern under room temperature is shown in figure 11B. In C_7 incubated media nearly 97% degradation occurred. C_4 and C_2 mineralized approximately 96% and 95% phenanthrene, respectively. The results are in line with Abed et al. [7] conclusion that the optimum temperature for consortium degradation of phenanthrene lies between 28 – 40 ^{o}C. As would be anticipated, consortium metabolism under low temperatures decreased remarkably (figure 11C). Approximately 68% and 58%

degradation respectively transpired in cultures of C_7 and C_4 after incubation at day 18. The lowest mineralization activity was recorded in C_2 inoculated samples. The average degradation in C_7 and C_4 inoculated samples appeared somewhat higher than C_2. Microbial population diversity accounted for this observed improved degradation. This finding underlines the possibility of achieving efficient decontamination via bioaugmentation with mixture of different strains [111]. Degradation of phenanthrene by bacterial consortium has been reported to reach 58% following one week incubation [62].

5.2.2. Pyrene degradation

Metabolism of pyrene by bacterial consortium, though the average mineralization dropped below 80%, showed enhanced degradation at 37 ^oC and emphasizes the capability of consortium to degrade recalcitrant hydrocarbons. C_7 metabolized 78% PAH (figure 12A). C_4 and C_2 biodegraded 64% and 51% pyrene. Pyrene could be metabolized by microbial consortium as high as 79% at 30 ^oC [163]. Apparently, biodegradation of pyrene suffered obvious reductions at 25 ^oC (figure12B). Bioavailability of the PAH presumably declined leading to limited microbial uptake and consequent mineralization. The maximum biodegradation observed was not more than 75% for C_7 consortium. C_2 metabolism yielded the least mineralization of 45%. At further reduced temperature (10 ^oC), bacterial metabolism shrunk. Interestingly however, all the consortia investigated elicited somewhat closed levels of residual PAH in cultures. Under this temperature, pyrene biodegradation mediated by all the consortia did not exceed 40%. The results tend to contradict the findings of Raquel et al. [164] that confirmed about 55% - 99% LMW and HMW PAHs depletion

at 5 ^{o}C - 15 ^{o}C. Figure 12C depicts consortia metabolism at 10 ^{o}C. Degradation of recalcitrant components of crude oil in community of marine environments by consortium lay emphasis on synergistic metabolic activity of individual strains in biodegradation [25].

5.3.3. Naphthalene degradation

Biodegradation of petroleum hydrocarbons such as naphthalene is shown be effective using consortium [165][166]. At mesothermic temperature, consortium metabolic activity resulted in 100% biodegradation of naphthalene in cultures of C_2, C_7 and C_4 (figure 13A). Similarly under room temperature, naphthalene was not undetected in cultures of C_7 and C_4. Consortium C_2 being the least diverse, degraded 95% naphthalene (figure 13B). A similar study executed by de Carvalho and da Fonseca [52] demonstrated bacterial mineralization of low molecular weight hydrocarbons between 15 ^{o}C – 28 ^{o}C. Conversely, naphthalene removal reduced in media incubated at 10 ^{o}C (figure 13C). The average amount of naphthalene degraded fell below 65%. C_7 mineralized about 72% naphthalene, and the least degradation was noticed in C_2 incubated flasks.

5.3. Biodegradation of PAHs by Isolates at varying pH

In this study, metabolism of PAHs by bacteria under different pH ranges was variable, with maximum degradation occurring at circum-neutral pH. Bacterial degradation of the PAHs improved at circum-neutral pH. Neutral to slightly acidic and alkaline media favor

microbial metabolism of PAHs [47][163]. The strains metabolic activity was however affected by both extremes of pH (5.0 and 9.0), leading to decreased mineralization. Interestingly, biodegradation did not halt at pH 5.0 and 9.0. PAHs degradation under extremes of pH is not impossible [163].

5.3.1. Phenanthrene degradation

As a LMW PAH, effective bacterial metabolism of phenanthrene is not uncommon [167]. In this study, *R qinshengi* and *B. fungorum* was found to have degraded over 90% of the initial 100 ppm phenanthrene (figure 14A). Following 10 days incubation, 99% of phenanthrene (100 ppm) were apparently metabolized by *Sphingobium sp* FB3 [163]. *Sphingobium yanoikuyae* LD29 degraded 92% phenanthrene at initial concentration of 50 ppm within 4 days [168]. The metabolism pattern of phenanthrene is evident in figure 14A. In the media inoculated with *P. mirabilis*, approximately 95% biodegradation occurred at day 18. *B. brevis* and *P. borealis* both mineralized not less than 96% phenanthrene. The results are in line with Wong et al. [45] conclusion that pH 7.5 favors phenanthrene degradation. Under neutral pH, the researchers observed that bacteria removed about 80% phenanthrene in 16 days.

Biodegradation of phenanthrene in acidic medium showed remarkable variation compared with neutral environment. Bacterial degradation activity reduced by several orders of magnitude. *P. borealis* and *B. fungorum* degraded the highest amount of PAH in acidic medium (figure 14B). The strains utilized 87% and 90% phenanthrene, respectively.

Quantitatively speaking, this metabolism is conspicuously below the average mineralized under pH 7.0. *P. mirabilis* and *R. qinshengi* metabolized nearly equal quantum of PAH, these strains activity accounted for about 80% dissipation of phenanthrene at the end of day 18. The lowest mineralization was realized in *Ralstoina sp* inoculated media. The general trend of reduction in the magnitude of biodegradation in acidic medium reiterates pH effects on bacterial growth, enzyme activity and metabolism [45][47][169]. For example, at pH 5.2, *Sphingomonas paucimobilis* degradation of phenanthrene reduced drastically compared to pH 7 [46].

Bacterial uptake and assimilation of PAH in alkaline medium appeared profoundly reduced with some degree of similarity to that of biodegradation in acidic conditions (figure 14C). Interestingly, compared with acidic the medium, *Ralstonia sp* metabolic activity increased tremendously in pH 9.0. The strain degraded not less than 75% phenanthrene. The maximum mineralization of nearly 81% was reckoned in *P. mirabilis* inoculated medium, residual PAH detected was 19.39 ppm. *P. borealis* and *R. qinshengi* degraded 78% phenanthrene. At pH 9.0, *Sphingobium sp* FB3 could degrade phenanthrene [163].

5.3.2. Pyrene degradation

Optimum degradation of pyrene could occur in medium with pH 7.0 [163]. Pyrene degradation at neutral pH is depicted in figure 15 (A-C). As visible from figure 15A, *B. fungorum* metabolized approximately 58% pyrene. This represented the highest mineralized realized in flasks incubated at neutral pH. Metabolic activity of *R. qinshengi*

resulted in depletion of about 56% pyrene. In *P. borealis* and *P. miribilis* incubated inoculated media, 40% PAH was decomposed. *Caulobacter sp, B. brevis* and *Ralstonia sp* each degraded below 35% pyrene.

Under acidic conditions, bacterial metabolism of pyrene appeared to have reduced for all the strains studied. This however excludes *P. borealis, B. fungorum* and *R. qinshengi.* The strains metabolism increased marginally resulting in approximately 48%, 59% and 61% biodegradation respectively of initial 100 ppm pyrene. *P. mirabilis* and *B. brevis* each degraded not less than 26% pyrene.

Biodegradation of recalcitrant PAHs under extreme alkaline conditions has been reported to be limited. Indeed, pyrene assimilation and metabolism declined dramatically for most of the strains investigated at pH 9.0. The degradation as low as 21% was observed in *Caulobacter sp* inoculated samples. Contrarily, the metabolism of *P. borealis* and *B. fungorum* enhanced compared to neutral pH. The strains degraded 56% and 62% pyrene, respectively. Similar degradation profile was reported in early studies [170]. Enhanced degradation of pyrene under alkaline medium has been documented [168][171]. The relatively improved degradation in alkaline medium could be ascribed to increased solubilization of PAHs by biosurfactants produced by bacteria, thereby elevating bioavailability and bacterial metabolism [49][50][172].

5.3.3. Naphthalene degradation

Naphthalene was not detected in the media incubated with *B. fungorum* and *R. qinshengi*, the strains mineralized 100% of the spiked naphthalene (figure 16A). Under neutral pH conditions, *Stroptomyces sp* degraded 99.14% naphthalene in a week [173]. Nearly 100% naphthalene mineralization was achieved in 48 hours using *Pseudomonas* strains [174]. *Caulobacter sp* and *Ralstonia sp* utilized not less than 93% naphthalene. The least degradation of about 87% was confirmed in *B. brevis* inoculated samples. Figure 16 (A-C) illustrates the trajectory of bacterial degradation of naphthalene over pH range 5.0-9.0.

Biodegradation of naphthalene under acidic conditions decreased by several orders of magnitude (figure 16B). The maximum degradation was however recorded in *R. qinshengi* inoculated flasks, the strain mineralized 93% naphthalene. *Rhodococcus* genera are versatile and adapt to degradation of low molecular weight hydrocarbons particularly, at pH 3-11[83]. *B. fungorum* decomposed approximately 89% naphthalene. Quantitatively, *P. mirabilis* and *P. borealis* utilized closely similar amounts of PAH (75%). Naphthalene degradation has been reported in extremely acidic soils of pH 2 [48]. The researchers recorded 50% naphthalene mineralization in 28 days [48].

Bacterial metabolism in alkaline samples followed comparatively similar reduction trend as under acidic medium. Extreme pH though significantly inhibited bacterial metabolic activity, did not completely stop biodegradation. Naphthalene degradation in alkaline media is depicted in figure 16C. The maximum biodegradation of 95.5% occurred in *R.*

qinshengi incubated media. *P. mirabilis* degraded the least amount of PAH. *P. borealis*, *Caulobacter sp* and *B. fungorum* metabolized above 80% naphthalene.

5.4. PAHs degradation by Consortium at varying pH

PAHs mineralization was noticed have elevated deploying consortia compared with bacterial isolates. Biodegradation decreased substantially at extremes of pH for low LMW PAHs. Strangely enough, pyrene degradation did not follow a definite pattern across the pH ranges investigated. This notwithstanding, consortium metabolism of pyrene was comparatively higher.

5.4.1. Phenanthrene biodegradation

Mineralization activity of C_2 and C_4 consortia resulted in nearly 97% disappearance of phenanthrene. In the medium incubated using $C_{7,}$ phenanthrene was completely degraded. The pattern of bacterial metabolism is shown in figure 17A. About 99.0% phenanthrene was dissipated in similar research that deployed microbial consortium [163]. In a related study, over 95% phenanthrene degradation was realized by deploying PAH-degradation microbial community [175]. Surprisingly, biodegradation of phenanthrene by C_7 and C_4 in acidic medium was not significantly different. The consortia degraded a little above 80% of the initial 100 ppm PAH. The consortium C_2 metabolized the lowest amount of phenanthrene (71%). Figure 17B sheds light on the trend in phenanthrene mineralization

over the entire incubation period. Consortia metabolic activity under alkaline conditions was somewhat closer to that of acidic environment. The consortia C_4 and C_7 degraded 80% and 79% phenanthrene respectively. As would be anticipated, the consortium C_2 mineralized the least amount of hydrocarbon. Figure 17C illustrates the consortia metabolic activity over varying incubation times. It is vital to emphasize that the average phenanthrene degraded in both acidic and alkaline media was approximately 87%.

5.4.2. Pyrene biodegradation

The consortium C_7 mineralized a little above 80% pyrene. The diversity of strains in the group probably accounted for the observed high degradation. Consortia C_4 biodegraded about 60%, while C_2 degraded less than 50% pyrene (figure 18A). In acidic medium, maximum of about 75% biodegradation transpired in C_7 inoculated cultures. Consortium C_4 mineralized not less than 70%, while C_2 degraded about 60% of the PAH (Figure 18B). Contrary to acidic conditions, in alkaline medium, consortia C_4 and C_7 degraded less than 60% pyrene. It is however worthy to pin-point that C_2 utilized the lowest amount of pyrene while C_7 degraded the maximum (figure 18C). Partial biodegradation of HMW PAHs such as pyrene by consortium is common [175].

5.4.3. Naphthalene biodegradation

Naphthalene was undetected in consortium inoculated samples incubated at pH 7.0. Bacterial consortium completely metabolize the initial 100 ppm naphthalene (figure 19A). Bacterial PAH metabolism in acidic medium is shown in figure 19B. Naphthalene degradation reduced significantly at extremes of pH but did not fall below 80%. The consortium C_7 metabolized the maximum of approximately 91% naphthalene. About 87% degradation was reckoned in C_4 incubated samples. The minimum mineralization was found in C_2 inoculated cultures as less than 80% naphthalene was utilized. The metabolic activity of the consortia in alkaline environments appeared closely similar (figure 19C). The consortia metabolized not less than 80% naphthalene. It is worthy to reiterate that C_2 degraded less naphthalene in acidic medium.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

Proteus mirabilis which is well known for its activity on industrial dyes but not PAHs [64][65], appeared to have metabolized 95% phenanthrene. In a similar vein, *B. brevis* utilized about 96.5% phenanthrene at 37 ^{o}C. Members in the genera *Brevibacillus* are also known to assimilate and degrade textile dyes [33][72][73]. Biodegradation under room temperature showed somewhat different bacterial metabolism. *Caulobacter sp* and *P. mirabilis* mineralized over 93% phenanthrene. *B. fungorum* and *R. qinshengi* degraded approximately 90% and 88% phenanthrene, respectively. When incubation was carried out at low temperatures (10 ^{o}C), PAH biodegradation did not exceed 60% for all the strains investigated. *B. fungorum and R. qinshengi* however degraded closer to 60% phenanthrene. The microbial consortia C_7 and C_4 biodegraded 68% and 58%, respectively.

In this study, *B. fungorum* and *R. qinshengi* degraded the maximum amounts of pyrene at mesothermic temperature. *B. fungorum* and *R. qinshengi* utilized nearly 56% and 58% pyrene, respectively. C_7 co-metabolized 78%, while C_4 and C_2 biodegraded over 50% pyrene. The degradation of pyrene at room temperature followed similar trajectory. The Only visible exception is the relatively enhanced performance of *B. fungorum* as the strain degraded 59% pyrene. There was a noticeable shift in bacterial metabolism of pyrene at 10

^{o}C. *B. fungorum* and *R. qinshengi* degraded about 33% and 37%, pyrene respectively, the remaining strains were unable to produce above 30% biodegradation.

Naphthalene was completely mineralized in *B. fungorum* and *R. qinshengi* inoculated media at 37 ^{o}C. For all strains studied at 25 ^{o}C, over 80% naphthalene was realized to have dissipated. Interestingly, the highest mineralization of approximately 94% was observed in *P. mirabilis* and *R. qinshengi* incubated media. At extreme low temperature (10 ^{o}C) however, bacterial metabolism shrunk significantly. In *B. fungorum* and *R. qinshengi* inoculated flasks 61% and 64% naphthalene was degraded, respectively. *P. borealis* degraded over 50% of PAH, while metabolism of naphthalene by *Caulobacter sp*, *B. brevis* and *Ralstonia sp* was marginally above 30%. At extremes of temperatures, certain bacteria maintain their metabolic activity and appreciably metabolize hydrocarbons [83].

Under neutral pH, 70% – 96% phenanthrene was mineralized by bacterial isolates. Bacterial consortia also biodegraded between 97% – 100% phenanthrene. Effective degradation of phenanthrene by consortium is possible [176]. In acidic medium, *P. borealis* and *B. fungorum* degraded 87% and 90% while in alkaline medium, approximately 81% biodeterioration was reckoned in *P. mirabilis* cultures, the remaining strains degraded below 80% phenanthrene.

It is important to note that *B. fungorum* and *R. qinshengi* showed unique degradation potentials under varying pH conditions. The strains mineralization was above 50% pyrene across the pH ranges investigated. The only notable exception was *R. qinshengi* metabolism which fell marginally below 30% in alkaline media. Pyrene degradation in *Sphingobium* strain FB3 is reported to occur at pH 5.0 – 9.0, with optimum pH being 7.0

[163]. Similar degradation profile was realized by Li et al. [177] using *Mycobacterium sp.* M11. Consortia cometabolism in neutral media yielded 50% – 80% loss of pyrene. The metabolism of *P. borealis* and *B. fungorum* reached maximum in pH 9.0 with the strains degrading between 56% - 62% pyrene. Consortia however degraded 57% - 68% pyrene. The optimum utilization of PAHs in C_7 cultures could be ascribed to the diverse nature of C_7 community [176].

Naphthalene mineralization range between 87% - 100% in neutral media. *B. fungorum* and *R. qinshengi* degraded 100% of the PAH. Naphthalene was completely biodegraded in flasks incubated with consortium. Maximum naphthalene degradation (>90%) was recorded in *R. qinshengi* inoculated flasks in both acidic and alkaline media. Consortium metabolism ranged between 80% - 91% loss of the PAH in pH 5.0, the optimum occurring in C_7 inoculated media.

6.2. Recommendations

Biodegradation of naphthalene, phenanthrene and pyrene by the 7 Bacterial strains and 3 Consortia did not halt at pH and temperatures selected. Further research capturing pH and temperatures outside the ranges (5.0 – 9.0) as well as (10 ^{o}C – 37 ^{o}C) is vital to elucidating PAHs mineralizing potentials of the bacterial strains in extreme environments.

Additional research would be required to establish whether the bacterial isolates produce toxic intermediate metabolites during PAHs degradation. Also, salinity impact on the bacterial isolates PAHs biodegradation requires further investigation.

In order to arrive at the best consortium suitable for bioremediation of the PAHs, different possible combinations of the strains must be trialed under separate pH and temperatures. Finally, the strains as well as consortia need to be trialed in the field (in situ) to understand their efficiency in degrading PAHs in the natural environment.

Appendix

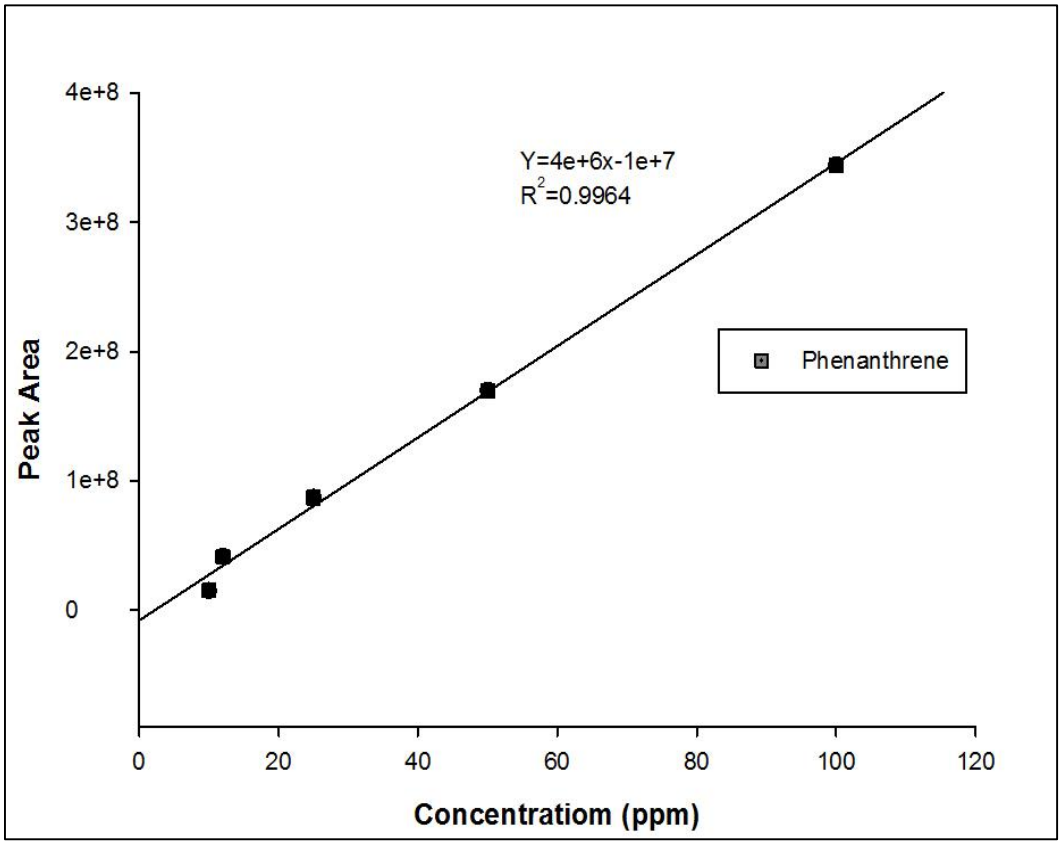

Figure 20 Phenanthrene calibration plot

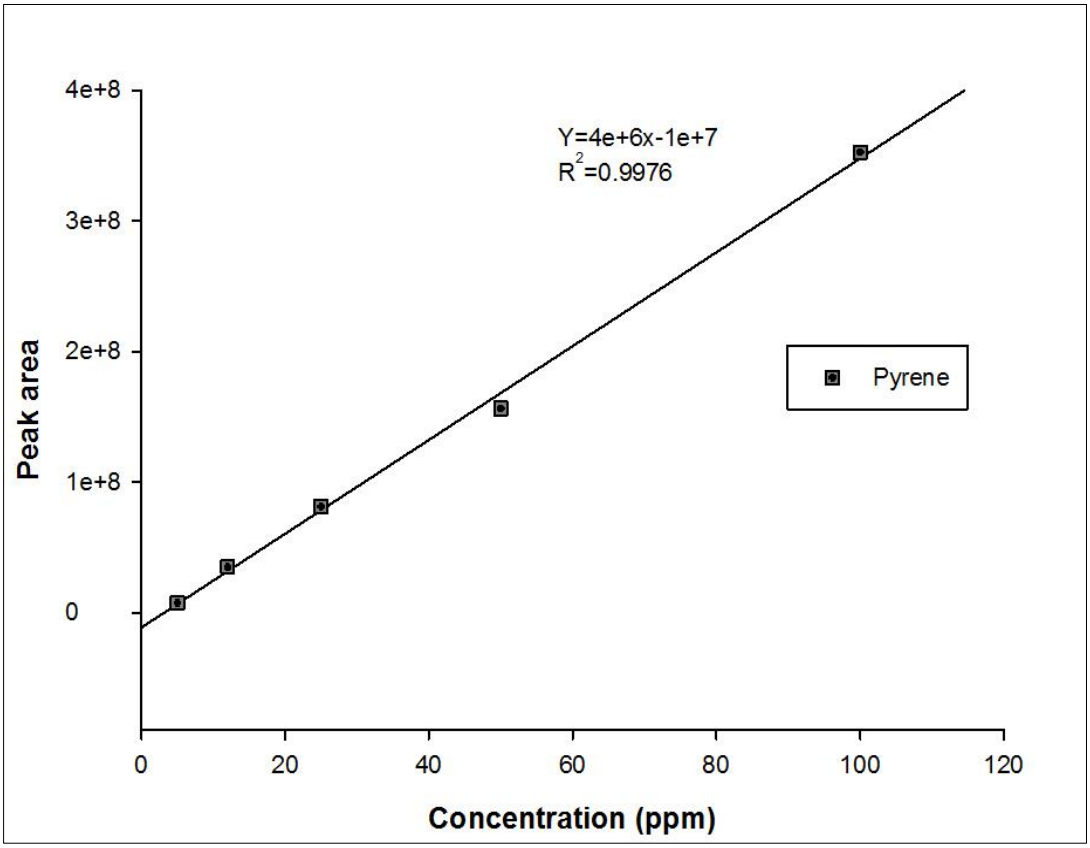

Figure 21 Pyrene calibration plot

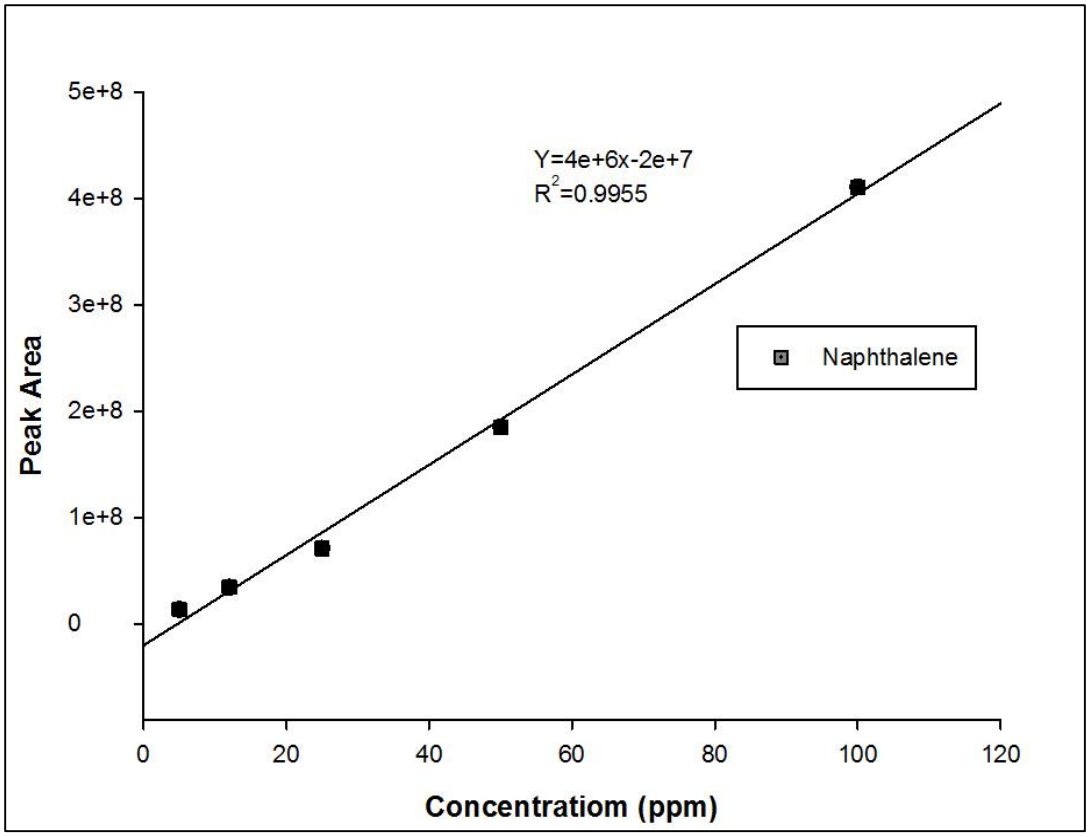

Figure 22 Naphthalene calibration plot

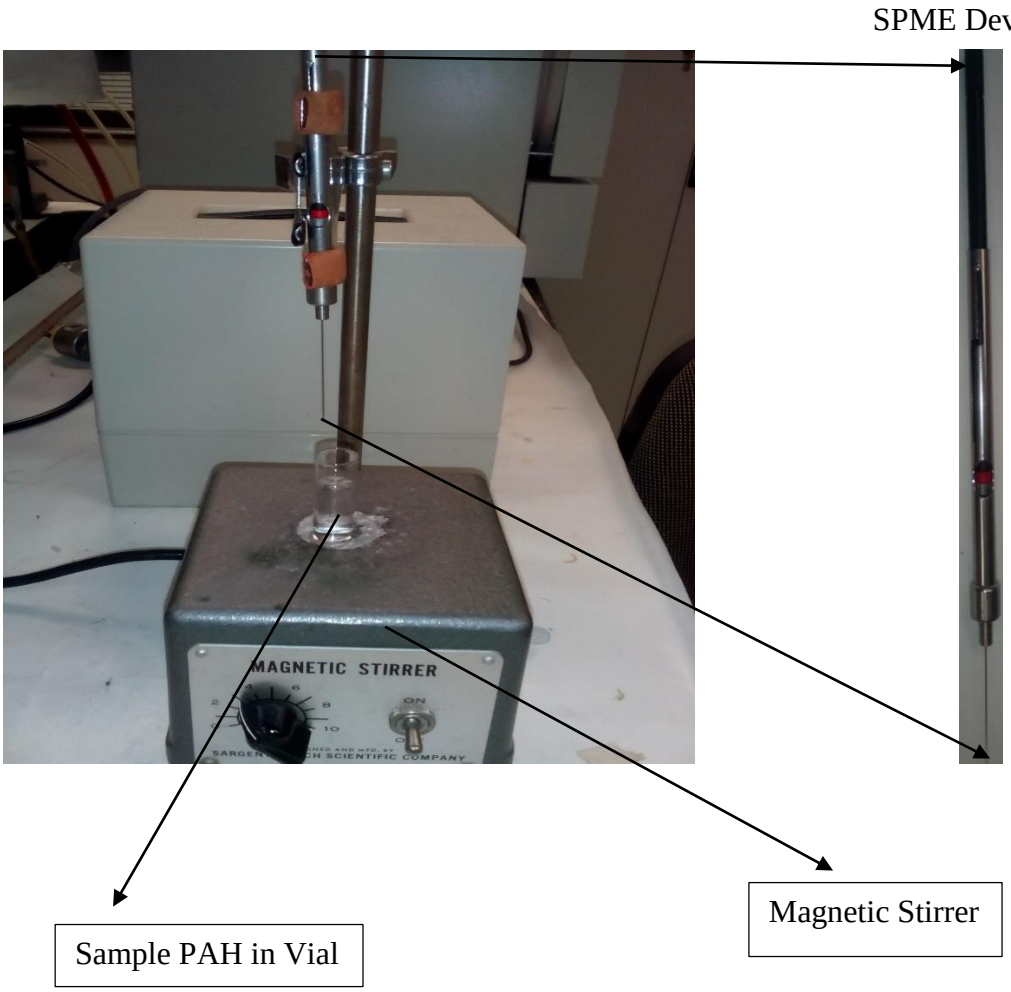

Figure 23 Schematics of SPME Set-up for PAHs extraction

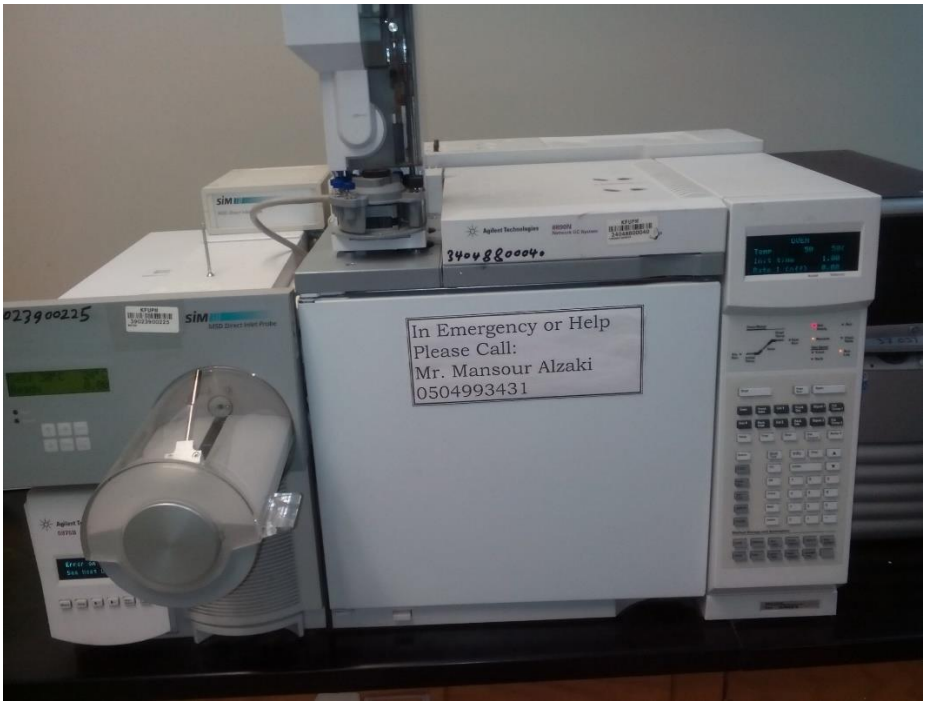

Figure 24 GC/MS

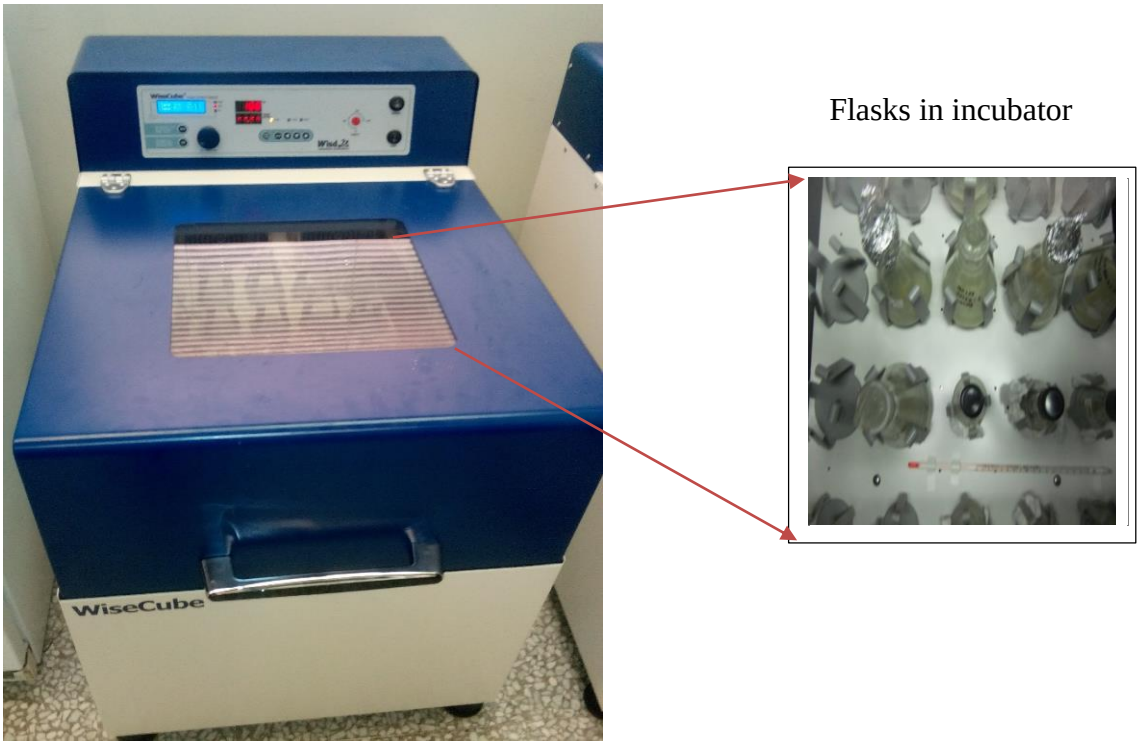

Figure 25 Samples in Rotary incubator

References

[1] E. Rosenberg, Ed., Microorganisms to Combat Pollution. *Dordrecht: Springer Netherlands*, 1993.

[2] Prince, Roger C. "Bioremediation of marine oil spills." *Trends in Biotechnology*, *vol*. 15, no. 5, pp 158 –160, 1997.

[3] S. Radwan, D. Al-Mailem, I. El-Nemr, and S. Salamah, "Enhanced remediation of hydrocarbon contaminated desert soil fertilized with organic carbons," *International Biodeteriorioration and Biodegradation*, vol. 46, no. 2, pp. 129–132, 2000.

[4] J. D. Van Hamme, A. Singh, and O. P. Ward, "Recent Advances in Petroleum Microbiology Recent Advances in Petroleum Microbiology," *Microbiology and molecular biology reviews*, vol. 67, no. 4, pp. 503 – 549, 2003.

[5] Röling, Wilfred FM, Michael G. Milner, D. Martin Jones, Francesco Fratepietro, Richard PJ Swannell, Fabien Daniel, and Ian M. Head. "Bacterial community dynamics and hydrocarbon degradation during a field-scale evaluation of bioremediation on a mudflat beach contaminated with buried oil." *Applied and environmental microbiology*, vol. 70, no. 5, pp. 2603–2613, 2004.

[6] D. Paul, G. Pandey, J. Pandey, and R. K. Jain, "Accessing microbial diversity for bioremediation and environmental restoration.," *Trends Biotechnology*, vol. 23, no. 3, pp. 135–42, 2005.

[7] R. Abed, “Bacterial diversity of a cyanobacterial mat degrading petroleum compounds at elevated salinities and temperatures,” *FEMS microbiology ecology*, vol. 57, no. 2, pp. 290–301, 2006.

[8] Al-Thukair, A. A., R. M. M. Abed, and L. Mohamed. "Microbial community of cyanobacteria mats in the intertidal zone of oil-polluted coast of Saudi Arabia."*Marine pollution bulletin*, vol. 54, no. 2, pp. 173–179, 2007.

[9] R. E. Countway, R. M. Dickhut, and E. A. Canuel, “Polycyclic aromatic hydrocarbon (PAH) distributions and associations with organic matter in surface waters of the York River, VA Estuary,” *Organic Geochemistry*, vol. 34, no. 2, pp. 209–224, Feb. 2003.

[10] Harvey, Ronald G. *Polycyclic aromatic hydrocarbons: chemistry and carcinogenicity*. CUP Archive, 1991.

[11] Ronald, W. Pickering. "Toxicity of polyaromatic hydrocarbons other than benzo (a) pyrene: a review." *Cutaneous and Ocular Toxicology*, vol. 19, no. 1, pp. 55-67, 2000.

[12] M. Engraff, C. Solere, K. E. C. Smith, P. Mayer, and I. Dahllöf, “Aquatic toxicity of PAHs and PAH mixtures at saturation to benthic amphipods: linking toxic effects to chemical activity.,” *Aquatic Toxicolology*, vol. 102, no. 3–4, pp. 142–9, 2011.

[13] E. Manoli, C. Samara, I. Konstantinou, and T. Albanis, “Polycyclic aromatic hydrocarbons in the bulk precipitation and surface waters of Northern Greece,” *Chemosphere*, vol. 41, no. 12, pp. 1845–1855, 2000.

[14] S. Froehner, M. Maceno, and K. S. Machado, “Predicting bioaccumulation of PAHs in the trophic chain in the estuary region of Paranagua, Brazil.,” *Environmental Monitoring and Assessment*, vol. 174, no. 1–4, pp. 135–45, 2011.

[15] S. Froehner, M. Maceno, E. C. Da Luz, D. B. Souza, and K. S. Machado, “Distribution of polycyclic aromatic hydrocarbons in marine sediments and their potential toxic effects.,” *Environmental Monitoring Assessment*, vol. 168, no. 1–4, pp. 205–13, 2010.

[16] Y. Ikenaka, M. Sakamoto, T. Nagata, H. Takahashi, Y. Miyabara, T. Hanazato, M. Ishizuka, T. Isobe, J.-W. Kim, and K.-H. Chang, “Effects of polycyclic aromatic hydrocarbons (PAHs) on an aquatic ecosystem: acute toxicity and community-level toxic impact tests of benzo[a]pyrene using lake zooplankton community.,” *Journal of Toxicological Science*, vol. 38, no. 1, pp. 131–6, 2013.

[17] J. P. Incardona, T. K. Collier, and N. L. Scholz, “Defects in cardiac function precede morphological abnormalities in fish embryos exposed to polycyclic aromatic hydrocarbons.,” *Toxicology and Applied Pharmacology*, vol. 196, no. 2, pp. 191–205, 2004.

[18] Yebra-Pimentel, Iria, Ricardo Fernández-González, Elena Martínez-Carballo, and Jesús Simal-Gándara. "A critical review about the health risk assessment of PAHs and their metabolites in foods." *Critical reviews in food science and nutrition* just-accepted (2013).

[19] E. B. Nilsen, R. J. Rosenbauer, C. C. Fuller, and B. J. Jaffe, "Sedimentary organic biomarkers suggest detrimental effects of PAHs on estuarine microbial biomass during the 20th century in San Francisco Bay, CA, USA," *Chemosphere*, vol. 119, pp. 961–970, 2015.

[20] K. T. Semple, K. J. Doick, L. Y. Wick, and H. Harms, "Microbial interactions with organic contaminants in soil: definitions, processes and measurement.," *Environmental Pollution*, vol. 150, no. 1, pp. 166–76, 2007.

[21] A K. Haritash and C. P. Kaushik, "Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review.," *Journal of Hazardous Materials*, vol. 169, no. 1–3, pp. 1–15, 2009.

[22] Reid, Brian J., Kevin C. Jones, and Kirk T. Semple. "Bioavailability of persistent organic pollutants in soils and sediments—a perspective on mechanisms, consequences and assessment." *Environmental Pollution* volk. 108, no. 1, pp. 103-112, 2000.

[23] Mackay, Donald, and Daryl Callcott. "Partitioning and physical chemical properties of PAHs." In PAHs and Related Compounds, *Springer Berlin Heidelberg*, pp. 325-345, 1998.

[24] Haines, J. R., M. Kadkhokayan, D. J. Mocsny, C. A. Jones, M. Islam, and A. D. Venosa. "Effect of salinity, oil type, and incubation temperature on oil degradation." *Applied Biotechnology for Site Remediation*, pp. 75-83, 1994.

[25] A. B. Salleh, F. M. Ghazali, R. Noor, Z. Abd, and M. Basri, "Bioremediation of Petroleum Hydrocarbon Pollution," *Indian journal of biotechnology*, vol. 2, no. pp. 411–425, 2003.

[26] A. P. Rowland, D. K. Lindley, G. H. Hall, M. J. Rossall, D. R. Wilson, D. G. Benham, A. F. Harrison, and R. E. Daniels, "Effects of beach sand properties , temperature and rainfall on the degradation rates of oil in buried oil / beach sand mixtures," *Environmental pollution*, vol. 109, no. 1 , pp. 109–118, 2000.

[27] G. L. Northcott and K. C. Jones, "Experimental approaches and analytical techniques for determining organic compound bound residues in soil and sediment.," *Environmental Pollution*, vol. 108, no. 1, pp. 19–43, 2000.

[28] S. Viamajala, B. M. Peyton, L. a Richards, and J. N. Petersen, "Solubilization, solution equilibria, and biodegradation of PAH's under thermophilic conditions.," *Chemosphere*, vol. 66, no. 6, pp. 1094–106, 2007.

[29] J. E. Stewart, "Effect of Four Dispersants on Biodegradation and Growth of Bacteria on Crude Oil Effect of Four Dispersants on Biodegradation and Growth of Bacteria on Crude Oil," *Applied microbiology*, vol. 28, no. 4, pp. 547-552, 1974.

[30] D. Delille, A. Dessommesb, and C. Rosiersc, “Influence of Daylight on Potential Biodegradation of Diesel and Crude Oil in Antarctic Seawater,” *Marine environmental research*, vol. 45, no. 3, pp. 249–258, 1998.

[31] D. Delille, E. Pelletier, A. Rodriguez-Blanco, and J.-F. Ghiglione, “Effects of nutrient and temperature on degradation of petroleum hydrocarbons in sub-Antarctic coastal seawater,” *Polar Biology*, vol. 32, no. 10, pp. 1521–1528, 2009.

[32] L. A. M. Ruberto, S. Vazquez, a. Lobalbo, and W. P. Mac Cormack, “Psychrotolerant hydrocarbon-degrading Rhodococcus strains isolated from polluted Antarctic soils,” *Antarctic Science*, vol. 17, no. 1, pp. 47–56, 2005.

[33] T. R. Waghmode, M. B. Kurade, A. N. Kabra, and S. P. Govindwar, “Degradation of Remazol Red dye by Galactomyces geotrichum MTCC 1360 leading to increased iron uptake in Sorghum vulgare and Phaseolus mungo from soil,” *Biotechnology and Bioprocess Engineering*, vol. 17, no. 1, pp. 117–126, 2012.

[34] Feitkenhauer, Heiko, Rudolf Müller, and Herbert MAuml. "Degradation of polycyclic aromatic hydrocarbons and long chain alkanes at 6070 C by Thermus and Bacillus spp." *Biodegradation*, vol. 14, no. 6, pp. 367–372, 2003.

[35] O. G. Brakstad and K. Bonaunet, “Biodegradation of petroleum hydrocarbons in seawater at low temperatures (0-5 degrees C) and bacterial communities associated with degradation.,” *Biodegradation*, vol. 17, no. 1, pp. 71–82, 2006.

[36] F. Coulon, B. a McKew, a M. Osborn, T. J. McGenity, and K. N. Timmis, “Effects of temperature and biostimulation on oil-degrading microbial communities in temperate estuarine waters.,” *Environmental Microbiology*, vol. 9, no. 1, pp. 177–86, 2007.

[37] E. Rosenberg, R. Legmann, A. Kushmaro, R. Taube, E. Adler, and E. Z. Ron, “Petroleum bioremediation ? a multiphase problem,” *Biodegradation*, vol. 3, no. 2–3, pp. 337–350, 1992.

[38] Swannell, Richard PJ, Barry C. Croft, Alyson L. Grant, and Kenneth Lee. "Evaluation of bioremediation agents in beach microcosms." *Spill Science and Technology Bulletin*, vol. 2, no. 2, pp. 151-159, 1995.

[39] G. D. Breedveld and M. Sparrevik, “Nutrient-limited biodegradation of PAH in various soil strata at a creosote contaminated site.,” *Biodegradation*, vol. 11, no. 6, pp. 391–9, 2000.

[40] H. I. Atagana, R. J. Haynes, and F. M. Wallis, “Optimization of soil physical and chemical conditions for the bioremediation of creosote-contaminated soil.,” *Biodegradation*, vol. 14, no. 4, pp. 297–307, 2003.

[41] F. Coulon, E. Pelletier, L. Gourhant, and D. Delille, “Effects of nutrient and temperature on degradation of petroleum hydrocarbons in contaminated sub-Antarctic soil.,” *Chemosphere*, vol. 58, no. 10, pp. 1439–48, 2005.

[42] D. Sarkar, M. Ferguson, R. Datta, and S. Birnbaum, “Bioremediation of petroleum hydrocarbons in contaminated soils: comparison of biosolids addition, carbon supplementation, and monitored natural attenuation.,” *Environmental Pollution*, vol. 136, no. 1, pp. 187–95, Jul. 2005.

[43] M. Nikolopoulou and N. Kalogerakis, “Biostimulation strategies for fresh and chronically polluted marine environments with petroleum hydrocarbons,” *Journal of chemical technology and biotechnology*, vol. 84, no. 6, pp. 802–807, 2009.

[44] Olajide, Patience O., and L. B. Ogbeifun. "Hydrocarbon biodegrading potentials of a Proteus vulgaris strain isolated from fish samples." *American Journal of Applied Sciences*, vol. 7, no. 7, pp. 922–928, 2010.

[45] Wong, J. W. C., K. M. Lai, C. K. Wan, K. K. Ma, and M. Fang. "Isolation and optimization of PAH-degradative bacteria from contaminated soil for PAHs bioremediation." *Water, Air, and Soil Pollution* vol. 139, no. 1-4, pp. 1-13, 2002.

[46] Kästner, Matthias, Maren Breuer-Jammali, and Bernd Mahro. "Impact of inoculation protocols, salinity, and pH on the degradation of polycyclic aromatic hydrocarbons (PAHs) and survival of PAH-degrading bacteria introduced into soil." *Applied and Environmental Microbiology* vol. 64, no. 1. pp. 359-362, 1998.

[47] Pawar, Rakesh Mahadev. "The effect of soil pH on degradation of polycyclic aromatic hydrocarbons." PhD dissertation, University of Hertfordshire, 2012.

[48] Stapleton, Raymond D., Dwayne C. Savage, Gary S. Sayler, and Gary Stacey. "Biodegradation of aromatic hydrocarbons in an extremely acidic environment."*Applied and environmental microbiology* vol. 64, no. 11, pp. 4180-4184, 1998.

[49] Lovaglio, Roberta B., Vinícius L. da Silva, Tulio de Lucca Capelini, Marcos N. Eberlin, Rudolf Hausmann, Marius Henkel, and Jonas Contiero. "Rhamnolipids Production by a Pseudomonas eruginosa LBI Mutant: Solutions and Homologs Characterization." *Tenside Surfactants Detergents* vol. 51, no. 5, pp. 397-405, 2014.

[50] G. Bai, M. L. Brusseau, and R. M. Miller, "Influence of cation type, ionic strength, and pH on solubilization and mobilization of residual hydrocarbon by a biosurfactant," *Journal of Contaminant Hydrology*, vol. 30, no. 3–4, pp. 265–279, 1998.

[51] Huijer, Keisha. "Trends in oil spills from tanker ships 1995-2004." *International Tanker Owners Pollution Federation (ITOPF), London* 30, 2005.

[52] C. C. C. R. de Carvalho and M. M. R. da Fonseca, "The remarkable Rhodococcus erythropolis.," *Applied Microbiology Biotechnology*, vol. 67, no. 6, pp. 715–26, 2005.

[53] C. Sakulthaew, S. Comfort, C. Chokejaroenrat, C. Harris, and X. Li, "A combined chemical and biological approach to transforming and mineralizing PAHs in runoff water.," *Chemosphere*, vol. 117, pp. 1–9, 2014.

[54] de Lorenzo, Victor. "Blueprint of an oil-eating bacterium." *Nature biotechnology*, vol. 24, no. 8, pp. 952-953, 2006.

[55] Brar, Satinder K., M. Verma, R. Y. Surampalli, K. Misra, R. D. Tyagi, N. Meunier, and J. F. Blais. "Bioremediation of hazardous wastes—a review."*Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management* vol. 10, no. 2, pp. 59-72, 2006.

[56] Mishra, Sanjeet, Jeevan Jyot, Ramesh C. Kuhad, and Banwari Lal. "Evaluation of inoculum addition to stimulate in situ bioremediation of oily-sludge-contaminated soil." *Applied and environmental microbiology* vol. 67, no. 4, pp. 1675-1681, 2001.

[57] M. Farhadian, D. Duchez, C. Vachelard, and C. Larroche, "Monoaromatics removal from polluted water through bioreactors-a review.," *Water Resources*, vol. 42, no. 6–7, pp. 1325–41, 2008.

[58] T. Gentry, C. Rensing, and I. Pepper, "New Approaches for Bioaugmentation as a Remediation Technology," *Critical reviews in environmental science and technology*, vol. 34, no. 5, pp. 447–494, 2004.

[59] K. S. M. Rahman, J. Thahira-Rahman, P. Lakshmanaperumalsamy, and I. M. Banat, "Towards efficient crude oil degradation by a mixed bacterial consortium.," *Bioresources Technolology*, vol. 85, no. 3, pp. 257–61, 2002.

[60] C. Alisi, R. Musella, F. Tasso, C. Ubaldi, S. Manzo, C. Cremisini, and A. R. Sprocati, “Bioremediation of diesel oil in a co-contaminated soil by bioaugmentation with a microbial formula tailored with native strains selected for heavy metals resistance.,” *Science of Total Environment*, vol. 407, no. 8, pp. 3024–32, 2009.

[61] X. Li, P. Li, X. Lin, C. Zhang, Q. Li, and Z. Gong, “Biodegradation of aged polycyclic aromatic hydrocarbons (PAHs) by microbial consortia in soil and slurry phases.,” *Journal of Hazardous Materials*, vol. 150, no. 1, pp. 21–6, 2008.

[62] S. Festa, B. M. Coppotelli, and I. S. Morelli, “Bacterial diversity and functional interactions between bacterial strains from a phenanthrene-degrading consortium obtained from a chronically contaminated-soil,” *International Biodeterioration and Biodegradation*, vol. 85, pp. 42–51, 2013.

[63] B. V. Mohite, S. P. Pawar, and A. Morankar, “Isolation, selection and biodegradation profile of phenol degrading bacteria from oil contaminated soil.,” *Bulletin of environmental contamination and toxicology*, vol. 87, no. 2, pp. 143–6, 2011.

[64] S. Mohana, C. Desai, and D. Madamwar, “Biodegradation and decolourization of anaerobically treated distillery spent wash by a novel bacterial consortium.,” *Bioresources Technollogy*, vol. 98, no. 2, pp. 333–9, 2007.

[65] O. D. Olukanni, A. A Osuntoki, D. C. Kalyani, G. O. Gbenle, and S. P. Govindwar, "Decolorization and biodegradation of Reactive Blue 13 by Proteus mirabilis LAG.," *Journal of Hazardous Materials*, vol. 184, no. 1–3, pp. 290–8, 2010.

[66] L. A Launen, J. Dutta, R. Turpeinen, M. E. Eastep, R. Dorn, V. H. Buggs, J. W. Leonard, and M. M. Häggblom, "Characterization of the indigenous PAH-degrading bacteria of Spartina dominated salt marshes in the New York/New Jersey Harbor.," *Biodegradation*, vol. 19, no. 3, pp. 347–63, 2008.

[67] M. Kumari, P. Ghosh, Swati, and I. S. Thakur, "Microcosmic study of endosulfan degradation by Paenibacillus sp. ISTP10 and its toxicological evaluation using mammalian cell line," *International Biodeterioration and Biodegradation*, vol. 96, pp. 33–40, 2014.

[68] Mohanty, Gita, and Suparna Mukherji. "Biodegradation rate of diesel range n-alkanes by bacterial cultures Exiguobacterium aurantiacum and Burkholderia cepacia." *International Biodeterioration and Biodegradation*, vol. 61, no. 3, pp. 240-250, 2008.

[69] Goris, Johan, Paul De Vos, Jesús Caballero-Mellado, Joonhong Park, Enevold Falsen, John F. Quensen, James M. Tiedje, and Peter Vandamme. "Classification of the biphenyl-and polychlorinated biphenyl-degrading strain LB400T and relatives as Burkholderia xenovorans sp. nov." *International Journal of Systematic and Evolutionary Microbiology* vol. 54, no. 5, pp. 1677-1681, 2004.

[70] Z. R. Suárez-Moreno, J. Caballero-Mellado, B. G. Coutinho, L. Mendonça-Previato, E. K. James, and V. Venturi, "Common features of environmental and potentially beneficial plant-associated Burkholderia.," *Microbial Ecology,* vol. 63, no. 2, pp. 249–66, 2012.

[71] M. Andreolli, S. Lampis, E. Zenaro, M. Salkinoja-Salonen, and G. Vallini, "Burkholderia fungorum DBT1: a promising bacterial strain for bioremediation of PAHs-contaminated soils.," *FEMS microbiology letters,* vol. 319, no. 1, pp. 11–8, 2011.

[72] M. B. Kurade, T. R. Waghmode, and S. P. Govindwar, "Preferential biodegradation of structurally dissimilar dyes from a mixture by Brevibacillus laterosporus.," *Journal of Hazardous Materials*, vol. 192, no. 3, pp. 1746–55, 2011.

[73] W. Lang, S. Sirisansaneeyakul, L. Ngiwsara, S. Mendes, L. O. Martins, M. Okuyama, and A. Kimura, "Characterization of a new oxygen-insensitive azoreductase from Brevibacillus laterosporus TISTR1911: Toward dye decolorization using a packed-bed metal affinity reactor.," *Bioresources Technology*, vol. 150C, pp. 298–306, 2013.

[74] J. Ye, H. Yin, H. Peng, J. Bai, D. Xie, and L. Wang, "Biosorption and biodegradation of triphenyltin by Brevibacillus brevis," *Bioresources Technology* vol. 129, pp. 236–241, 2013.

[75] J. Dalal, P. M. Sarma, M. Lavania, A. K. Mandal, and B. Lal, “Evaluation of bacterial strains isolated from oil-contaminated soil for production of polyhydroxyalkanoic acids (PHA),” *Pedobiologia (Jena).*, vol. 54, no. 1, pp. 25–30, 2010.

[76] Becher, Dörte, Michael Specht, Elke Hammer, Wittko Francke, and Frieder Schauer. "Cometabolic Degradation of Dibenzofuran by Biphenyl-CultivatedRalstonia sp. Strain SBUG 290." *Applied and environmental microbiology*, vol. 66, no. 10, pp. 4528-4531, 2000.

[77] H. Ishida and K. Nakamura, “Trichloroethylene degradation by Ralstonia sp. KN1-10A constitutively expressing phenol hydroxylase: transformation products, NADH limitation, and product toxicity.,” *Journal of Bioscience and Bioengineering*, vol. 89, no. 5, pp. 438–45, 2000.

[78] Kahng, Hyung-Yeel, Armando M. Byrne, Ronald H. Olsen, and Jerome J. Kukor. "Characterization and role of tbuX in utilization of toluene by Ralstonia pickettii PKO1." *Journal of bacteriology*, vol. 182, no. 5, pp. 1232-1242, 2000.

[79] M. P. Ryan, J. T. Pembroke, and C. C. Adley, “Ralstonia pickettii in environmental biotechnology: potential and applications,” *Journal of Applied Microbiology*, vol. 103, no. 4, pp. 754–64, 2007.

[80] J. Li, J. Liu, W. Shen, X. Zhao, Y. Hou, H. Cao, and Z. Cui, “Isolation and characterization of 3,5,6-trichloro-2-pyridinol-degrading Ralstonia sp. strain T6.,” *Bioresources Technolology*, vol. 101, no. 19, pp. 7479–83, 2010.

[81] S.-K. Lee and S. B. Lee, “Isolation and characterization of a thermotolerant bacterium Ralstonia sp. strain PHS1 that degrades benzene, toluene, ethylbenzene, and o -xylene,” *Applied Microbiological Biotechnology*, vol. 56, no. 1–2, pp. 270–275, 2001.

[82] M. J. Larkin, L. A Kulakov, and C. C. R. Allen, “Biodegradation and Rhodococcus--masters of catabolic versatility.,” *Current Opinion in Biotechnolology*, vol. 16, no. 3, pp. 282–90, 2005.

[83] C. C. C. R. de Carvalho, “Adaptation of Rhodococcus erythropolis cells for growth and bioremediation under extreme conditions.,” *Research in microbiology*, vol. 163, no. 2, pp. 125–36, 2012.

[84] C. C. C. R. de Carvalho and M. M. R. da Fonseca, “Degradation of hydrocarbons and alcohols at different temperatures and salinities by Rhodococcus erythropolis DCL14.,” *FEMS Microbiology Ecolology*, vol. 51, no. 3, pp. 389–99, 2005.

[85] D. Dean-Ross, J. D. Moody, J. P. Freeman, D. R. Doerge, and C. E. Cerniglia, “Metabolism of anthracene by a Rhodococcus species.,” *FEMS Microbiology Letters*, vol. 204, no. 1, pp. 205–11, 2001.

[86] L. Huang, T. Ma, D. Li, F. Liang, R.-L. Liu, and G. Li, “Optimization of nutrient component for diesel oil degradation by Rhodococcus erythropolis.,” *Marine Pollution Bulletin*, vol. 56, no. 10, pp. 1714–8, 2008.

[87] E. Quek, Y. P. Ting, and H. M. Tan, “Rhodococcus sp. F92 immobilized on polyurethane foam shows ability to degrade various petroleum products.,” *Bioresources Technology*, vol. 97, no. 1, pp. 32–8, 2006.

[88] X. Song, Y. Xu, G. Li, Y. Zhang, T. Huang, and Z. Hu, “Isolation, characterization of Rhodococcus sp. P14 capable of degrading high-molecular-weight polycyclic aromatic hydrocarbons and aliphatic hydrocarbons.,” *Marine Pollution Bulletin*, vol. 62, no. 10, pp. 2122–8, 2011.

[89] Jouanneau, Yves, Christine Meyer, Jean Jakoncic, Vivian Stojanoff, and Jacques Gaillard. "Characterization of a naphthalene dioxygenase endowed with an exceptionally broad substrate specificity toward polycyclic aromatic hydrocarbons." *Biochemistry*, vol. 45, no. 40, pp. 12380-12391, 2006.

[90] Kafilzadeh, Farshid, Parvin Amiri, Abbasali Rezaeian Jahromi, and Niloofar Mojoodi. "Isolation and molecular identification of fluoranthene degrading bacteria from the mangrove sediments in South of Iran." *International Journal of* Bioscience, vol. 3, no. 5, pp. 60-67, 2013.

[91] F. Kafilzadeh, A. Rezaei, M. Javad, and N. Nejad, “RTICLE Evaluation of phenanthrene biodegradation by indigenous bacteria isolated from mangrove sediments in the Persian Gulf,” *Advances in Environmental Biology*, vol. 7, no. 9, pp. 2218–2224, 2013.

[92] B. Kumari, S. N. Singh, F. Deeba, M. Sharma, V. Pandey, and D. P. Singh, “Elucidation of Pyrene Degradation Pathway in Bacteria,” *Advances in Bioresearch*, vol. 4, no. 2, 2013.

[93] R. Margesin, C. Moertelmaier, and J. Mair, “Low-temperature biodegradation of petroleum hydrocarbons (n-alkanes, phenol, anthracene, pyrene) by four actinobacterial strains,” *International Biodeterioration and Biodegradation*, vol. 84, pp. 185–191, 2013.

[94] M. Dhote, A. Juwarkar, A. Kumar, G. S. Kanade, and T. Chakrabarti, “Biodegradation of chrysene by the bacterial strains isolated from oily sludge,” *World Journal of Microbiology and Biotechnology*, vol. 26, no. 2, pp. 329–335, 2009.

[95] Dave, Bharti P., Chirag M. Ghevariya, Jwalant K. Bhatt, Dushyant R. Dudhagara, and Rahul K. Rajpara. "Enhanced biodegradation of total polycyclic aromatic hydrocarbons (TPAHs) by marine halotolerant Achromobacter xylosoxidans using Triton X-100 and β-cyclodextrin–A microcosm approach."*Marine pollution bulletin*, vol. 79, no. 1, pp. 123-129, 2014.

[96] F. Kafilzadeh, N. Sehatnezhad, E. Kadivar, and Rezaeian AA, Bioremediation of Chrysene by Isolated Bacteria From Petroleum Polluted Soils In Iran,” vol. 1, no. 8, pp. 1047–1056, 2012.

[97] T. Pantsyrnaya, S. Delaunay, J.-L. Goergen, E. Guédon, C. Paris, P. Poupin, E. Guseva, and J. Boudrant, “Biodegradation of Phenanthrene by Pseudomonas putida and a Bacterial Consortium in the Presence and in the Absence of a Surfactant.,” *Indian Journal of Microbiology*, vol. 52, no. 3, pp. 420–6, 2012.

[98] Sopeña, Fatima, Leonila Laiz, Esmeralda Morillo, Maria Antonia Sanchez-Trujillo, Jaime Villaverde, Valme Jurado, and Cesareo Saiz-Jimenez. "Phenanthrene biodegradation by Pseudomonas xanthomarina isolated from an aged contaminated soil." *Clean–Soil, Air, Water* vol. 42, no. 6, pp. 785-790, 2014.

[99] Vaidehi, Khobragade, and S. D. Kulkarni. “Microbial Remediation of Polycyclic Aromatic Hydrocarbons : An Overview,” *Research Journal of Chemistry and Environment*, vol. 16, no. 4, p. 2012, 2012.

[100] Díaz-Ramírez, Ildefonso, Erika Escalante-Espinosa, Randy Adams Schroeder, Reyna Fócil-Monterrubio, and Hugo Ramírez-Saad. "Hydrocarbon Biodegradation Potential of Native and Exogenous Microbial Inocula in Mexican Tropical Soils." 2013.

[101] T. Hadibarata and R. A. Kristanti, “Identi fi cation of Metabolic Intermediates in Microbial Degradation of Chrysene by Armillaria sp F022,” *Indonesian Journal of Biotechnology* vol. 15, no. 2, pp. 46–53, 2013.

[102] Hadibarata, Tony, and Risky Ayu Kristanti. "Effect of surfactants and identification of metabolites on the biodegradation of fluoranthene by basidiomycetes fungal isolate Armillaria sp. F022." *Bioprocess and biosystems engineering* vol. 37, no. 4, pp. 593-600, 2014.

[103] Y. Li, H. Wang, F. Hua, M. Su, and Y. Zhao, "Trans-membrane transport of fluoranthene by Rhodococcus sp. BAP-1 and optimization of uptake process," *Bioresources Technolology*, vol. 155, pp. 213–219, 2014.

[104] S. E. Agarry and M. O. Aremu, "Batch Equilibrium and Kinetic Studies of Simultaneous Adsorption and Biodegradation of Naphthalene by Orange Peels Immobilized Pseudomonas aeruginosa NCIB 950," *Journal of Bioremediation and Biodegradegradation*, vol. 03, no. 2, 2012.

[105] C. Balachandran, V. Duraipandiyan, K. Balakrishna, and S. Ignacimuthu, "Petroleum and polycyclic aromatic hydrocarbons (PAHs) degradation and naphthalene metabolism in Streptomyces sp. (ERI-CPDA-1) isolated from oil contaminated soil.," *Bioresources Technology*, vol. 112, pp. 83–90, 2012.

[106] C. Lin, L. Gan, and Z.-L. Chen, "Biodegradation of naphthalene by strain Bacillus fusiformis (BFN).," *Journal of Hazardous Materials*, vol. 182, no. 1–3, pp. 771–7, 2010.

[107] Liu, Zhongbao, Annette M. Jacobson, and Richard G. Luthy. "Biodegradation of naphthalene in aqueous nonionic surfactant systems." *Applied and environmental microbiology* 61, no. 1, pp. 145-151, 1995.

[108] C. Muangchinda, R. Pansri, W. Wongwongsee, and O. Pinyakong, “Assessment of polycyclic aromatic hydrocarbon biodegradation potential in mangrove sediment from Don Hoi Lot, Samut Songkram Province, Thailand.,” *Journal of Applied Microbiology*, vol. 114, no. 5, pp. 1311–24, 2013.

[109] F. Moscoso, F. J. Deive, M. a Longo, and M. a Sanromán, “Technoeconomic assessment of phenanthrene degradation by Pseudomonas stutzeri CECT 930 in a batch bioreactor.,” *Bioresources Technolology*, vol. 104, pp. 81–9, 2012.

[110] A. G. Ali and A. A. El-hanafy, “Isolation and Identification of Three-Rings Polyaromatic Hydrocarbons (Anthracene and Phenanthrene) Degrading Bacteria,” *Am-Eur Journal of Agriculture and Environmental Science*, vol. 5, no. 1, pp. 31–38, 2009.

[111] V. Patel, J. Patel, and D. Madamwar, “Biodegradation of phenanthrene in bioaugmented microcosm by consortium ASP developed from coastal sediment of Alang-Sosiya ship breaking yard.,” *Marine Pollution Bulletin*, vol. 74, no. 1, pp. 199–207, 2013.

[112] Mishra, S., and S. N. Singh. "Biodegradation of benzo (a) pyrene mediated by catabolic enzymes of bacteria." *International Journal of Environmental Science and Technology*, vol. 11, no. 6, pp. 1571-1580, 2014.

[113] H. Peng, H. Yin, J. Deng, J.-S. Ye, S.-N. Chen, B.-Y. He, and N. Zhang, “Biodegradation of Benzo[a]pyrene by Arthrobacter oxydans B4,” *Pedosphere*, vol. 22, no. 4, pp. 554–561, 2012.

[114] Puangkaew, Jiraporn, Gullaya Wattayakorn, Chompunuch Virunanon, Warawut Chulalaksananukul, and Suphang Chulalaksananukul. "Biodegradation of Benzo (a) pyrene by Marine Denitrifying Bacteria." *Journal of Selcuk University Natural and Applied Science*, pp. 22-31, 2013.

[115] X. Lu, T. Zhang, H. Han-Ping Fang, K. M. Y. Leung, and G. Zhang, "Biodegradation of naphthalene by enriched marine denitrifying bacteria," *International Biodeterioration Biodegradation*, vol. 65, no. 1, pp. 204–211, 2011.

[116] Y. Wang, R. Wan, S. Zhang, and S. Xie, "Anthracene biodegradation under nitrate-reducing condition and associated microbial community changes," *Biotechnology and Bioprocess Engineering*, vol. 17, no. 2, pp. 371–376, 2012.

[117] Liu, Ming, Yongliang Cui, Yousheng Duan, Jiangchun Zhong, Wei Sun, Min Wang, Shangzhong Liu, and Qing X. Li. "Synthesis of metabolites of polycyclic aromatic hydrocarbons." *Mini-Reviews in Organic Chemistry* vol. 7, no. 2, pp. 134-144, 2010.

[118] Seo, Jong-Su, Young-Soo Keum, and Qing X. Li. "Bacterial degradation of aromatic compounds." *International journal of environmental research and public health*, vol. 6, no. 1, pp. 278-309, 2009.

[119] Kanaly, Robert A., Richard Bartha, Kazuya Watanabe, and Shigeaki Harayama. "Rapid mineralization of benzo [a] pyrene by a microbial consortium growing on diesel fuel." *Applied and Environmental Microbiology*, vol. 66, no. 10, pp. 4205-4211, 2000.

[120] Sabaté, J., M. Grifoll, M. Viñas, and A. M. Solanas. "Isolation and characterization of a 2-methylphenanthrene utilizing bacterium: identification of ring cleavage metabolites." *Applied microbiology and biotechnology*, vol. 52, no. 5, pp. 704-712, 1999.

[121] S. Gao, J.S. Seo, J. Wang, Y.-S. Keum, J. Li, and Q. X. Li, "Multiple degradation pathways of phenanthrene by Stenotrophomonas maltophilia C6.," *International Biodeterioration and Biodegradation*, vol. 79, pp. 98–104, 2013.

[122] Habe, Hiroshi, and Toshio Omori. "Genetics of polycyclic aromatic hydrocarbon metabolism in diverse aerobic bacteria." *Bioscience, biotechnology, and biochemistry,* vol. 67, no. 2, pp. 225-243, 2003.

[123] Seo, Jong-Su, Young-Soo Keum, Yuting Hu, Sung-Eun Lee, and Qing X. Li. "Phenanthrene degradation in Arthrobacter sp. P1-1: initial 1, 2-, 3, 4-and 9, 10-dioxygenation, and meta-and ortho-cleavages of naphthalene-1, 2-diol after its formation from naphthalene-1, 2-dicarboxylic acid and hydroxyl naphthoic acids." *Chemosphere*, vol. 65, no. 11, pp. 2388-2394, 2006.

[124] Mallick, Somnath, Subhankar Chatterjee, and Tapan K. Dutta. "A novel degradation pathway in the assimilation of phenanthrene by Staphylococcus sp. strain PN/Y via meta-cleavage of 2-hydroxy-1-naphthoic acid: formation of trans-2, 3-dioxo-5-(2′-hydroxyphenyl)-pent-4-enoic acid." *Microbiology*, vol. 153, no. 7, pp. 2104-2115, 2007.

[125] Laurie, Andrew D., and Gareth Lloyd-Jones. "The phn Genes of Burkholderiasp. Strain RP007 Constitute a Divergent Gene Cluster for Polycyclic Aromatic Hydrocarbon Catabolism." *Journal of bacteriology,* vol. 181, no. 2, pp. 531-540, 1999.

[126] Laurie, Andrew D., and Gareth Lloyd-Jones. "Conserved and hybrid meta-cleavage operons from PAH-degrading Burkholderia RP007." *Biochemical and biophysical research communications,* vol. 262, no. 1, pp. 308-314, 1999.

[127] Kang, H., S. Y. Hwang, Y. M. Kim, E. Kim, Y.S. Kim, S-K. Kim, S. W. Kim, C. E. Cerniglia, K. L. Shuttleworth, and G. J. Zylstra. "Degradation of phenanthrene and naphthalene by a Burkholderia species strain." *Canadian journal of microbiology*, vol. 49, no. 2, pp. 139-144, 2003.

[128] J.S. Seo, Y.S. Keum, Y. Hu, S.-E. Lee, and Q. X. Li, "Phenanthrene degradation in Arthrobacter sp. P1-1: initial 1,2-, 3,4- and 9,10-dioxygenation, and meta- and ortho-cleavages of naphthalene-1,2-diol after its formation from naphthalene-1,2-dicarboxylic acid and hydroxyl naphthoic acids.," *Chemosphere*, vol. 65, no. 11, pp. 2388–94, 2006.

[129] J.S. Seo, Y.S. Keum, Y. Hu, S.-E. Lee, and Q. X. Li, "Degradation of phenanthrene by Burkholderia sp. C3: initial 1,2- and 3,4-dioxygenation and meta- and ortho-cleavage of naphthalene-1,2-diol.," *Biodegradation*, vol. 18, no. 1, pp. 123–31, 2007.

[130] Moody, Joanna D., James P. Freeman, Daniel R. Doerge, and Carl E. Cerniglia. "Degradation of phenanthrene and anthracene by cell suspensions of Mycobacterium sp. strain PYR-1." *Applied and Environmental Microbiology*, vol. 67, no. 4, pp. 1476-1483, 2001.

[131] C. E. Cerniglia, "Biodegradation of polycyclic aromatic hydrocarbons," *Current Opinion in Biotechnology*, vol. 4, no. 3, pp. 331–338, 1993.

[132] H. Habe and T. Omori, "Genetics of polycyclic aromatic hydrocarbon metabolism in diverse aerobic bacteria.," *Bioscience, biotechnology, and biochemistry* , vol. 67, no. 2, pp. 225–43, 2003.

[133] Morasch, Barbara, Eva Annweiler, Rolf J. Warthmann, and Rainer U. Meckenstock. "The use of a solid adsorber resin for enrichment of bacteria with toxic substrates and to identify metabolites: degradation of naphthalene, o-, and m-xylene by sulfate-reducing bacteria." *Journal of microbiological methods*, vol. 44, no. 2, pp. 183-191, 2001.

[134] D. Dean-Ross and C. E. Cerniglia, "Degradation of pyrene by Mycobacterium flavescens," *Applied Microbiology and Biotechnology*, vol. 46, no. 3, pp. 307–312, 1996.

[135] Kim, Yong-Hak, James P. Freeman, Joanna D. Moody, Karl-Heinrich Engesser, and Carl E. Cerniglia. "Effects of pH on the degradation of phenanthrene and pyrene by Mycobacterium vanbaalenii PYR-1." *Applied microbiology and biotechnology*, vol. 67, no. 2, pp. 275-285, 2005.

[136] Liang, Yanna, Dale R. Gardner, Charles D. Miller, Dong Chen, Anne J. Anderson, Bart C. Weimer, and Ronald C. Sims. "Study of biochemical pathways and enzymes involved in pyrene degradation by Mycobacterium sp. strain KMS." *Applied and environmental microbiology*, vol. 72, no. 12, pp. 7821-7828, 2006.

[137] Y.H. Kim, J. P. Freeman, J. D. Moody, K.-H. Engesser, and C. E. Cerniglia, "Effects of pH on the degradation of phenanthrene and pyrene by Mycobacterium vanbaalenii PYR-1.," *Applied microbiology and biotechnology*, vol. 67, no. 2, pp. 275–85, 2005.

[138] Vila, Joaquim, Zaira López, Jordi Sabaté, Cristina Minguillón, Anna M. Solanas, and Magdalena Grifoll. "Identification of a novel metabolite in the degradation of pyrene by Mycobacterium sp. strain AP1: actions of the isolate on two-and three-ring polycyclic aromatic hydrocarbons." *Applied and Environmental Microbiology*, vol. 67, no. 12, pp. 5497-5505, 2001.

[139] M. Bouchez, D. Blanchet, and J.P. Vandecasteele, "Degradation of polycyclic aromatic hydrocarbons by pure strains and by defined strain associations: inhibition phenomena and cometabolism," *Applied microbiology and biotechnology*, vol. 43, no. 1, pp. 156–164, 1995.

[140] K. L. Shuttleworth and E. Cerniglia, "Environmental aspects of PAH biodegradation," *Applied Biochemistry and Biotechnology*, vol. 54, no. 1–3, pp. 291–302, 1995.

[141] S. Lundstedt, *Analysis of PAHs and their transformation products in contaminated soil and remedial processes*. 2003.

[142] Kochany, J., and R. J. Maguire. "Abiotic transformations of polynuclear aromatic hydrocarbons and polynuclear aromatic nitrogen heterocycles in aquatic environments." *Science of the total environment*, vol. 144, no. 1, pp. 17-31, 1994.

[143] R. van Herwijnen, B. Joffe, A. Ryngaert, M. Hausner, D. Springael, H. a J. Govers, S. Wuertz, and J. R. Parsons, "Effect of bioaugmentation and supplementary carbon sources on degradation of polycyclic aromatic hydrocarbons by a soil-derived culture.," *FEMS Microbiology Ecology*, vol. 55, no. 1, pp. 122–35, 2006.

[144] F. Z. Ferradji, S. Mnif, A. Badis, S. Rebbani, D. Fodil, K. Eddouaouda, and S. Sayadi, "Naphthalene and crude oil degradation by biosurfactant producing Streptomyces spp. isolated from Mitidja plain soil (North of Algeria)," *International Biodeterioration and Biodegradation*, vol. 86, pp. 300–308, 2014.

[145] Pawliszyn, Janusz. "Theory of solid-phase microextraction." *Journal of chromatographic science*, vol. 38, no. 7, pp. 270-278, 2000.

[146] Lord, Heather, and Janusz Pawliszyn. "Evolution of solid-phase microextraction technology." *Journal of Chromatography*, vol. 885, no. 1, pp. 153-193, 2000.

[147] G. Ouyang and J. Pawliszyn, "A critical review in calibration methods for solid-phase microextraction.," *Analitca chimica acta*, vol. 627, no. 2, pp. 184–97, 2008.

[148] A. J. King, J. W. Readman, and J. L. Zhou, “Determination of polycyclic aromatic hydrocarbons in water by solid-phase microextraction–gas chromatography–mass spectrometry,” *Analitca chimica acta*, vol. 523, no. 2, pp. 259–267, Oct. 2004.

[149] A. Pedetta, K. Pouyte, M. K. Herrera Seitz, P. a. Babay, M. Espinosa, M. Costagliola, C. a. Studdert, and S. R. Peressutti, “Phenanthrene degradation and strategies to improve its bioavailability to microorganisms isolated from brackish sediments,” *International Biodeterioration and Biodegradation*, vol. 84, pp. 161–167, 2013.

[150] M. C. Deng, J. Li, F. R. Liang, M. Yi, X. M. Xu, J. P. Yuan, J. Peng, C. F. Wu, and J. H. Wang, “Isolation and characterization of a novel hydrocarbon-degrading bacterium Achromobacter sp. HZ01 from the crude oil-contaminated seawater at the Daya Bay, southern China,” *Marine. Pollution Bulletin*, vol. 83, no. 1, pp. 79–86, 2014.

[151] A. L. Juhasz, “Pyrene degradation and metabolite formation by Burkholderia cepacia strain VUN 10,003,” *Australasian Biotechnology*, vol. 7, no. 6, pp. 350–354, 1997.

[152] K. Rehmann, H. P. Noll, C. E. W. Steinberg, and A. A. Kettrup, “Pyrene degradation by Mycobacterium sp. strain KR2,” *Chemosphere*, vol. 36, no. 14, pp. 2977–2992, 1998.

[153] S. N. Singh, B. Kumari, S. K. Upadhyay, S. Mishra, and D. Kumar, “Bacterial degradation of pyrene in minimal salt medium mediated by catechol dioxygenases: Enzyme purification and molecular size determination,” *Bioresources Technology*, vol. 133, pp. 293–300, 2013.

[154] W. Xia, Z. Du, Q. Cui, H. Dong, F. Wang, P. He, and Y. Tang, “Biosurfactant produced by novel Pseudomonas sp. WJ6 with biodegradation of n-alkanes and polycyclic aromatic hydrocarbons,” *Journal of Hazardous Materials*, vol. 276, pp. 489–498, 2014.

[155] Rehmann, Klaus, Harald P. Noll, Christian EW Steinberg, and Antonius A. Kettrup. "Pyrene degradation by Mycobacterium sp. strain KR2." *Chemosphere*, vol. 36, no. 14, pp. 2977–2992, 1998.

[156] K. Chen, Q. Zhu, Y. Qian, Y. Song, J. Yao, and M. M. F. Choi, “Microcalorimetric investigation of the effect of non-ionic surfactant on biodegradation of pyrene by PAH-degrading bacteria Burkholderia cepacia,” *Ecotoxicology and Environmental Safety*, vol. 98, pp. 361–367, 2013.

[157] J.S. Seo, Y.S. Keum, R. M. Harada, and Q. X. Li, “Isolation and characterization of bacteria capable of degrading polycyclic aromatic hydrocarbons (PAHs) and organophosphorus pesticides from PAH-contaminated soil in Hilo, Hawaii.,” *Journal of agricultural and food chemistry*, vol. 55, no. 14, pp. 5383–9, 2007.

[158] Q. Li, G. Hou, A. Lin, X. Yang, L. Wei, and S. Xiong, “Degradation of pyrene by immobilized PW bacteria,” *Beijing Huagong Daxue Xuebao (Ziran Kexueban)/Journal Beijing University Chemical Technology. (Natural Sci. Ed.*, vol. 40, pp. 36–40, 2013.

[159] Z. C. Teh and T. Hadibarata, “Enhanced Degradation of Pyrene and Metabolite Identification by Pleurotus eryngii F032,” *Water, Air, Soil Pollution*, vol. 225, no. 5, p. 1909, 2014.

[160] J. Zhang, X. Zhang, J. Liu, R. Li, and B. Shen, “Isolation of a thermophilic bacterium, Geobacillus sp. SH-1, capable of degrading aliphatic hydrocarbons and naphthalene simultaneously, and identification of its naphthalene degrading pathway,” *Bioresources Technology*, vol. 124, pp. 83–89, 2012.

[161] A. Tøndervik, P. Bruheim, L. Berg, T. E. Ellingsen, H. K. Kotlar, S. Valla, and M. Throne-Holst, “Ralstonia sp. U2 naphthalene dioxygenase and Comamonas sp. JS765 nitrobenzene dioxygenase show differences in activity towards methylated naphthalenes,” *Journal of Bioscience and Bioengineering*, vol. 113, no. 2, pp. 173–178, 2012.

[162] S. Bisht, P. Pandey, G. Kaur, H. Aggarwal, a. Sood, S. Sharma, V. Kumar, and N. S. Bisht, “Utilization of endophytic strain bacillus sp. SBER3 for biodegradation of polyaromatic hydrocarbons (PAH) in soil model system,” *European Journal of Soil Biology*, vol. 60, pp. 67–76, 2014.

[163] B. Fu, Q. X. Li, T. Xu, Z. L. Cui, Y. Sun, and J. Li, “Sphingobium sp. FB3 degrades a mixture of polycyclic aromatic hydrocarbons,” *International Biodeterioration and Biodegradation*, vol. 87, pp. 44–51, 2014.

[164] R. Simarro, N. González, L. F. Bautista, and M. C. Molina, “Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by a wood-degrading consortium at low temperatures,” *FEMS Microbiology Ecology*, vol. 83, no. 2, pp. 438–449, 2013.

[165] A. PRAKASH, S. BISHT, J. SINGH, P. TEOTIA, R. KELA, and V. KUMAR, “Biodegradation potential of petroleum hydrocarbons by bacteria and mixed bacterial consortium isolated from contaminated sites,” *Turkish Journal of Engineering and Environment Science*, vol. 38, no. 1, pp. 41–50, 2014.

[166] N. Xu, M. Bao, P. Sun, and Y. Li, “Study on bioadsorption and biodegradation of petroleum hydrocarbons by a microbial consortium.,” *Bioresources Technology*, vol. 149, pp. 22–30, 2013.

[167] J.-S. Seo, Y.-S. Keum, and Q. X. Li, “Bacterial degradation of aromatic compounds.,” *International Journal of Environment, Resources and Public Health*, vol. 6, no. 1, pp. 278–309, 2009.

[168] F. Liu, J. S. Liang, Y. Sun, Y. Bin Guo, Q. X. Li, and J. Li, “Isolation and degradation characteristics of a HMW PAHs-degrading strain LD29,” *Huanjing Kexue/Environmental Science.*, vol. 32, no. 6, pp. 1799–1804, 2011.

[169] M. Lin, X. Hu, W. Chen, H. Wang, and C. Wang, “Biodegradation of phenanthrene by Pseudomonas sp. BZ-3, isolated from crude oil contaminated soil,” *International Biodeterioration and Biodegradation*, vol. 94, pp. 176–181, 2014.

[170] L. Ping, C. Zhang, C. Zhang, Y. Zhu, H. He, M. Wu, T. Tang, Z. Li, and H. Zhao, “Isolation and characterization of pyrene and benzo[a]pyrene-degrading Klebsiella pneumonia PL1 and its potential use in bioremediation.,” *Applied Microbiological Biotechnology*, vol. 98, no. 8, pp. 3819–28, 2014.

[171] Y. L. Du, P. D. Tai, Q. F. Shi, and P. J. Li, “Identification and degrading characteristics of PAHs-degrading bacterial strain X20,” *Chinese Journal of Ecology*, vol. 29, no. 6, pp. 1208–1212, 2010.

[172] H. Chang, M. Nie, B. Ge, C. Liu, Q. Yang, L. Zhou, X. Fan, C. Sun, R. Wei, and X. Jiang, “Effects of rhamnolipid on oil degradation by Pseudomonas aeruginosa strain NY3,” *Chinese Journal of Environmental Engineering*, vol. 7, no. 2, pp. 771–776, 2013.

[173] C. Balachandran, V. Duraipandiyan, K. Balakrishna, and S. Ignacimuthu, “Petroleum and polycyclic aromatic hydrocarbons (PAHs) degradation and naphthalene metabolism in Streptomyces sp. (ERI-CPDA-1) isolated from oil contaminated soil,” *Bioresources Technology*, vol. 112, pp. 83–90, 2012.

[174] P. Isaac, L. a. Sánchez, N. Bourguignon, M. E. Cabral, and M. a. Ferrero, “Indigenous PAH-degrading bacteria from oil-polluted sediments in Caleta

Cordova, Patagonia Argentina," *International Biodeterioration and Biodegradation*, vol. 82, pp. 207–214, 2013.

[175] H. P. Bacosa and C. Inoue, "Polycyclic aromatic hydrocarbons (PAHs) biodegradation potential and diversity of microbial consortia enriched from tsunami sediments in Miyagi, Japan," *Journal of Hazardous. Materials.*, vol. 283, pp. 689–697, 2015.

[176] M. Wu, L. Chen, Y. Tian, Y. Ding, and W. a. Dick, "Degradation of polycyclic aromatic hydrocarbons by microbial consortia enriched from three soils using two different culture media," *Environmental Pollution*, vol. 178, pp. 152–158, 2013.

[177] Li, Q. X., B. Q. Fan, M. B. Gong, and Hui Yang. "[Isolation, identification of a pyrene-degrading strain Mycobacterium sp. M11 and its degrading characteristics]." *Huan jing ke xue= Huanjing kexue/[bian ji, Zhongguo ke xue yuan huan jing ke xue wei yuan hui" Huan jing ke xue" bian ji wei yuan hui.]* vol. 29, no. 3, pp. 763-768, 2008.

[178] Bushnell and Haas, *Journal of Bacteriology*, vol. 41, pp. 653, 1941.

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